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EFFECT OF INBREEDING ON EST-1 POLYMORPHISM AND MATING ACTIVITY IN TWO SPECIES OF *DROSOPHILA BIPECTINATA* COMPLEX

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Est-1 polymorphism and mating activity were studied in three populations each of *D. malerkotliana* and *D. parabipectinata* of the *D. bipictinata* complex and after ten generations of maintenance in the laboratory. Both *D. malerkotliana* and *D. parabipectinata* of the *D. bipectinata* complex showed insignificant variation for Est-1 polymorphism within base populations, within the F_{10} generation and also between base populations and the F_{10} generation indicating the role of balancing selection in the maintenance of enzyme polymorphism in both natural and laboratory conditions. Both *D. malerkotliana* and *D. parabipectinata* showed significant difference in mating activity after maintenance in the laboratory for 10 generations.

(*Key words:* Est-1 polymorphism, mating activity, *Drosophila malerkotliana; Drosophila parabipectinata*)

INTRODUCTION

Natural populations of most organisms possess large stores of genetic variation which may be related to ecological differentiation. Several workers have attempted to study the mechanisms involved in maintaining polymorphism. Evidence has been provided by RICHMOND (1972) and by AYALA *et al.* (1972) that the allelic variation in nature is maintained by balancing selection, while MARINKOVIC & AYALA (1975 a, b) have shown that fitness components are affected by the allozyme variants.

Natural populations and laboratory strains of the same species show significant interpopulational variability in mating propensity and copulation duration (KAUL & PARSONS, 1965; SANCHEZ & BLANCO, 1989). In *Drosophila,* selection expeiiments have been carried out for mating speed in different species and significant results have often been obtained (MANNING, 1961, 1963; KESSLER, 1968, 1969; SINGH & CHATTERJEE, 1988a). Mating propensity has been found to be associated with inversion polymorphism (eg. SINGH & CHATTERJEE, 1988b), metric tiaits (EWING, 1964; HEGDE & NASEERULLA, 1992) and fertility (PRAKASH, 1967). PASCUAL *et al.* (1990) have shown that in *D. subobscura* mating activity increases with the time of maintenance in the laboratory while in *D. simulans* inbreeding decreases mating propensity (RINGO *et al.,* 1987). In *D. melanogaster* mating ability was not greatly affected by laboratory culture (KOHANE & PARSONS, 1987) and allele frequencies were restabilised around values characteristic of the original wild-caught populations (PALABOST-CHARLES, 1982). Therefore it is obvious that different species of *Drosophila* respond

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to inbreeding in different ways. Hence an attempt has been made to investigate and report the effect of inbreeding on Est-1 polymorphism and mating activity in two species of *Drosophila bipectinata* complex, viz. *Drosophila malerkotliana* and *Drosophila parabipectinata.*

MATERIALS AND METHODS

Chromosomally monomorphic populations of *D. malerkotliana* and *D. parabipectinata* were used in the present study. The three populations of *D. malerkotliana* used were from Baroda (BA), Mahadeswara Hills (MH), both of Indian origin, and Clayton (CN) of Australia, while the three populations of *D. parabipectinata* were from Varanasi (VN), Mysore (MS), both of India, and Clayton (CN) of Australia. Isofemale lines for each of these populations were maintained separately and the progeny derived from each line was inbred (full-sib mating) for 10 generations under laboratory conditions. Males of the base population and an equal number of females in the F_1, and some of the flies derived from the 10th generation, were used for the analysis of Est-1 polymorphism. Some of the flies derived from the progeny of base population and some of the flies of 10th generation were subjected to analysis of mating activity.

Est-1 polymorphism was studied by polyacrylamide gel electrophoretic technique. The gel slabs were prepared using solutions made as described by DAVIS (1964). The single fly homogenates were separately loaded in the sample slots above the large pore gel, and electrophoresis was carried out for 2 hours at 4° C. After electrophoresis, the gels were stained for esterase using histochemical stain (AYALA, 1972) with 1-naphthyl acetate as the substrate. When bands appeared on the gels, they were transferred to 7% acetic acid.

The fast-moving band was designated as F, and the slow-moving one as S. Individuals with both the bands were treated as heterozygotes. Allelic frequencies were calculated by adding half of the total number of heterozygotes observed to the homozygotes scored, and this value was divided by the total number of genomes sampled. Heterozygosity per individual (H) was calculated using the formula (SINGH & COULTHART, 1982):

$$H = 1 - \Sigma Pi^2$$

where Pi is the frequency of the 'i'th allele and the summation is over all the alleles present in the locus sampled. Heterogeneity in allelic frequencies was calculated by using G-statistics (SOKAL & ROHLF, 1981).

To study the mating activity in the base population and F_{10} generation, sexes were separated within 4 h of eclosion, maintained separately and aged for 5 days. In the laboratory, to study copulation duration and mating propensity, 10 males and 10 females were together introduced into a glass mating chamber without anaesthesia and allowed to acclimatize to the mating chamber for 30 seconds. Then copulation initiation time (the time elapsed until mating of the first pair from 30 seconds after mixing the males and females) was recorded. When mating occurred, pairs in copulation were removed with an aspirator without disturbing the mating and transferred to individual vials with food to record the copulation duration of each pair. Observations continued for one hour and the number of pairs that had mated at the end of one hour was noted (mating propensity). For each population a total of five replicates was observed, and all the observations were carried out at 24 ± 1° C.

RESULTS

The allelic frequencies at Est–1 locus in both base population and the F_{10} generation of *D. malerkotliana* and *D. parabipectinata* are given in Tables 1 and 2, respectively. Both, the base population and F_{10} generation, show insignificant difference (by G–statistics) for both F and S alleles, and also for heterozygosity per individual in both *D. malerkotliana* and *D. parabipectinata*. When the allelic frequencies of the three base populations are compared with the frequencies of their respective F_{10} generation, the difference is found to be insignificant.

Tables 3 and 4 provide the data on average time elapsed until mating of the first pair from the time of mixing the males and females of *D. malerkotliana* and *D. parabipectinata*, respectively. Analysis of variance computed for copulation initiation time of the three different populations each of *D. malerkotliana* and *D. parabipectinata* shows insignificant difference both among base populations and F_{10} generations (base population F = 0.16, F_{10} generation

TABLE 1. Allelic frequencies of Est–1 locus in three base populations of *D. malerkotliana* and at Generation 10 of laboratory maintenance.

Allele	Base population				F_{10} generation			
	Baroda (N=60)	Mahadeswara Hills (N=60)	Clayton (N=60)	G-value	Baroda (N=60)	Mahadeswara Hills (N=40)	Clayton (N=40)	G-value
S	0.45	0.62	0.52	1.65	0.43	0.50	0.52	0.71
F	0.55	0.38	0.48	1.90	0.57	0.50	0.48	1.64
H	0.49	0.47	0.50	1.14	0.49	0.50	0.50	1.19

N = Number of genomes sampled.
G value not significant at 5% level.

TABLE 2. Allelic frequencies of Est–1 locus in three base populations of *D. parabipectinata* and at Generation 10 of laboratory maintenance.

Allele	Base population				F_{10} generation			
	Varanasi (N=60)	Mysore (N=40)	Clayton (N=60)	G-value	Varanasi (N=60)	Mysore (N=40)	Clayton (N=40)	G-value
S	0.53	0.52	0.47	1.35	0.55	0.48	0.50	0.94
F	0.47	0.48	0.53	0.51	0.45	0.52	0.50	1.48
H	0.50	0.50	0.49	1.19	0.49	0.50	0.50	1.19

N = Number of genomes sampled.
G value not significant at 5% level.

TABLE 3. Mating activity in the base populations of *D. malerkotliana* and at Generation 10 of laboratory maintenance. (Values are mean ± SE)

	Base population				F_{10} generation			
	Baroda	Mahadeswara Hills	Clayton	F value	Baroda	Mahadeswra Hills	Calyton	F value
Average time (min) elapsed until mating of first pair	3.78 ± 1.97	2.49 ± 0.49	3.07 ± 1.42	0.16	8.26 ± 3.20	5.62 ± 1.59	3.90 ± 0.50	0.69
Copulation duration (min)	12.55 ± 1.40	12.29 ± 1.30	13.42 ± 0.76	0.82	9.07 ± 0.25	11.31 ± 0.52	9.63 ± 0.34	5.82*
Mating %	52.00	58.00	52.00	0.13	16.00	42.00	42.00	13.06*

* $P < 0.01$.

TABLE 4. Mating activity in the base populations of *D. parabipectinata* and at Generation 10 of laboratory maintenance. (Values are mean ± SE).

	Base population				F_{10} generation			
	Varanasi	Mysore	Clayton	F value	Varanasi	Mysore	Clayton	F value
Average time (min) elapsed until mating of first pair	0.72 ± 0.32	1.12 ± 0.55	0.88 ± 0.21	0.21	4.12 ± 1.58	0.85 ± 0.34	3.81 ± 0.84	2.34
Copulation duration (min)	10.51 ± 0.36	10.60 ± 0.33	8.76 ± 0.36	8.22**	7.47 ± 0.28	10.06 ± 0.31	7.57 ± 0.18	26.16**
Mating %	78.00	68.00	68.00	0.64	48.00	74.00	52.00	6.12*

* $P < 0.05$; ** $P < 0.01$.

F = 0.69, and base population F = 0.21, F_{10} generation F = 2.34, respectively, for *D. malerkotliana* and *D. parabipectinata*).

Copulation duration in *D. malerkotliana* (Table 3), by ANOVA, shows insignificant variation among the three base populations (F = 0.82), while it is significantly different among the F_{10} generations (F = 5.82; $P < 0.01$).

Table 4 gives the data on copulation duration in *D. parabipectinata*. ANOVA shows that there is significant difference in the copulation duration between the base populations (F=8.22; $P<0.01$) as well as among the F_{10} generations (F = 26.16; $P < 0.01$).

Perusal of Tables 3 and 4 shows that the copulation duration has decreased signi-

ficantly in the F_{10} generation compared to the respective base population, in both *D. malerkotliana* and *D. parabipectinata*.

With regard to the mating propensity of *D. malerkotliana*, after arcsine transformation of the mating percentages, ANOVA shows insignificant difference between the three base populations ($F = 0.13$) while the difference is significant between the three populations after 10 generations of inbreeding ($F = 5.82$; $P < 0.01$, Table 3). If mating propensity of the F_{10} generation is compared with that of the respective base population, there is a significant decrease in the mating speed of BA ($t = 2.80$; $P < 0.05$) and MH ($t = 3.37$; $P < 0.01$) populations, while it is not significant in CN population ($t = 0.85$).

In *D. parabipectinata*, mating propensity does not show significant variation among the base populations ($F = 0.64$), but it is significant in the F_{10} generation ($F = 6.12$; $P < 0.05$). In VN population of *D. parabipectinata*, there is a significant decrease in the mating speed after its maintenance in the laboratory for 10 generations ($t=3.99$; $P < 0.01$).

DISCUSSION

Natural populations of *Drosophila* have been shown to have a high variability of esterase allozyme alleles (ASLUND & RASMUSON, 1976). In laboratory population studies on Est–6 in *D. melanogaster*, rapid gene frequency changes have occurred (MACINTYRE & WRIGHT, 1966; RASMUSON *et al.*, 1967) and stable equilibrium frequencies were reached after a few generations and maintained for at least 100 generations (ASLUND & RASMUSON, 1976). In our experiments we have found that the allelic frequency of S and F allele of Est–1 show insignificant difference between different populations of *D. malerkotliana* and *D. parabipectinata*. This confirms the view that the esterase locus is highly variable in different populations of some species, while less variable in others.

The F_{10} generations of *D. malerkotliana* and *D. parabipectinata* do not show significant difference in allele frequency either among themselves or when compared with their respective base population, indicating the role of balancing selection in the maintenance of enzyme polymorphism. Similar results were obtained by PRAKASH *et al.* (1969) who state that some form of balancing selection, dependent chiefly upon physiological properties of the polypeptides and only weekly on variation in the environment, is involved.

In both *D. malerkotliana* and *D. parabipectinata*, average time elapsed until mating of the first pair does not show significant variation between the base population and F_{10} generation, which means that it does not change significantly as a result of maintenance in the laboratory.

Copulation duration is not statistically significant in the three natural populations of *D. malerkotliana*, corroborating the view of HEGDE & KRISHNAMURTHY (1979) who showed that copulation duration does not differ significantly in different populations of *D. malerktoliana*. However, after a period of maintenance in the laboratory, copulation duration becomes significantly different in different isofemale lines of *D. malerkotliana*. Some authors (EHRMAN, 1963; MOURAD, 1965) studying *D. pseudoobscura* strains set up by Dr. M. Vetukhiv found genetic differences produced by the time of maintenance of the flies in the laboratory. This might also explain the significant difference obtained in the copulation duration of both base population ad F_{10} generation of *D. parabipectinata* strains in our study. As a consequence of increase

in mating activity, copulation duration has decreased significantly in the F_{10} generation both in *D. malerkotliana* and *D. parabipectinata*.

The statistical insignificance in the mating propensity in the base populaion of both *D. malerkotliana* and *D. parabipectinata* and its statistical significance in the F_{10} generation might again be an indication that mating activity changes with the time of maintenance under laboratory conditions. In *D. pseudoobscura*, ANDERSON (1966) observed non-significant genetic divergence in body size among the populations kept for 1.5 years. When the same populations were 6 years old, he found genetic divergence and this divergence was more striking when the populations were about 12 years old.

Mating propensity in both *D. malerkotliana* and *D. parabipectinata* decreases significantly in the F_{10} generation. This might be due to inbreeding depression. When individual inbred lines were assessed at progressively increasing levels of homozygosity, the decline in male fitness was almost continuous in *D. melanogaster* (SHARP, 1984). In our experiments, on an average, the mating speed has decreased by 2.07% per generation in *D. malerkotliana* while in *D. parabipectinata* the decrease is by 1.73% per generation. FALCONER (1981) suggests that characters close to fitness should exhibit little additive genetic variation but should suffer greatly from inbreeding depression. FULKER (1966) quoting MATHER argues that the genetic architecture of inbred lines may be regarded as a vestigial form of that found in natural populations inspite of considerable natural selection during inbreeding. Therefore individuals of a natural population show a high proportion of fast mating when compared to individuals of a laboratory population.

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BEAN CONDITIONING BY *CALLOSOBRUCHUS RHODESIANUS* (PIC.) AND *CALLOSOBRUCHUS MACULATUS* (F): THEIR EFFECTS ON OVIPOSITION

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Experiments were designed to determine whether conditioning of beans by adults is a factor affecting egg-laying in the bean weevils *Callosobruchus rhodesianus* (P.c.) and *Callosobruchus maculatus* (F.). Conditioning of seeds, in this study refers to the deposition of oviposition deterring compounds by adults whilst walking over the seeds. A variety of experimental treatments of self-conditioning of beans (homotypic) and conditioning by another species (heterotypic) demonstrated that the two species of bruchids respond differently in oviposition. Both species laid fewer eggs on conditioned grain compared with clean grain. The results indicate that egg-laying of *C. rhodesianus* is more affected by conditioning by *C. maculatus* than by homotypic conditioning, while *C. maculatus* is affected more by homotypic conditioning than by *C. rhodesianus*.

(*Key words*: *Callosobruchus rhodesianus*, *C. maculatus*, homotypic, heterotypic, conditioning)

INTRODUCTION

Many factors affect the number of eggs laid by females in the genus *Callosobruchus*. For example, the number of host seeds available to each female (CREDLAND, 1986), characteristics of host seeds such as roughness of seed–coats (NWANZE & HORBER, 1976) seed size and shape (NWANZE *et al.*, 1975), temperature and humidity (HOWE & CURRIE, 1964; GIGA & SMITH, 1983) and density of adult beetles (BELLOWS, 1982) all contributed to variations in oviposition rate. There are also numerous reports that the eggs are distributed evenly among seeds (eg. MITCHELL, 1975; Avidov *et al.*, 1965; GOKHALE & SRIVASTAVA, 1975). For internally-feeding granivorous insects such as the bean weevils a single ovipositing female will maximize her fitness by dispersing her eggs over the available seeds to minimise the effects of larval competition between her offspring (SMITH & LESSELLS, 1985; WILSON, 1988). Such oviposition behaviour has been shown to be mediated by an oviposition-marking pheromone (oviposition deterrent) (WASSERMAN, 1981, 1985; MITCHELL, 1975).

Two questions that arise are: (i) whether or not adults (males or females) 'condition' the beans to prevent others from exploiting the resource or (ii) whether the oviposition deterrent is released during egglaying only. YOSHIDA (1961, 1966) suggested the presence of a 'biological conditioning substance' which functions to mark beans on which adults have been crawling. He referred to the marking of beans as 'conditioning' and also tried to explain interspecific competition between *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.) in terms of the biological conditioning substance. The objectives of this study was to determine: (i) whether conditioning of the seed by males and virgin adults (male or female) were factors affecting egg laying and (ii) whether both *Callosobruchus rhodesianus*

(Pic.) and *C. maculatus* would respond to the conditioning of the other species.

MATERIALS AND METHODS

Origin and maintenance of beetles: Stock cultures of *C. rhodesianus* and *C. maculatus* came from the Natural Resources Institute, Storage Department, Slough. The insects were collected from cowpeas in Swaziland (*C. rhodesianus*) and Brazil ('Campinas' strain of *C. maculatus*) in the mid 1970s and were maintained in the laboratory on a Californian cultivar of blackeye beans ever since. All cultures were kept in a constant temperature and humidity (CTH) room at 27° C and 70% relative humidity (RH).

Experimental protocols: Oviposition and egg hatch on beans conditioned by males: Seventy-five newly emerged males (<1 day old) of *C. maculatus C. rhodesianus* were separately introduced into tubes containing ten blackeye beans (*Vigna unguiculata* (L.) Walp.) and allowed to wander over the beans for two days. Clean beans (unconditioned) were used as controls. The insects were removed and three mated females of either species were introduced in the three types of tube. Each of the six experimental combinations was replicated eight times. Ten days after the death of the females, the numbers of hatched and unhatched eggs were counted. The experiment was carried out in a CTH room at 27° C and 70% RH.

Oviposition on beans conditioned by virgin adults : In this experiment beans conditioned by either virgin males or virgin females of *C. maculatus* and *C. rhodesianus* were used. To obtain virgin insects, cowpeas with single prominent "windows" were removed from cultures and isolated in small tubes. The tubes were constantly observed and beetles sexed as they emerged. The beetles were sexed in the adult stage using sexually dimorphic characters. *C. maculatus* adults were sexed by examining the elytral pattern; the female is darkly coloured and always possessing four elytra spots characteristic of the species, in contrast to the male which is pale-brownish in colour and less distinctly coloured (SOUTHGATE *et al.*, 1957). *C. rhodesianus* beetles were sexed using colour characteristics; the female elytron is black at the base with either black apical half or dark medio-lateral area and black margins. No such markings are found in the males (SOUTHGATE, 1958).

Two virgin adults of the same sex were placed in tubes containing two blackeye beans for two days in a CTH room at 30°C and 70% RH. The adults were then removed and two mated females were introduced onto the conditioned grain. There were four combinations of conditioning by sex and each treatment was replicated fifteen times. Ten days after the females had died, the total number of eggs laid were counted.

RESULTS AND DISCUSSION

Oviposition and egg hatch on beans conditioned by males: The analysis of variance of the square root transformation of the total number of eggs laid showed that both *C. maculatus* and *C. rhodesianus* were significantly affected by conditioning ($P<0.01$).

The square root transformation was used to normalise the data. YOSHIDA (1966) referred to self-conditioning of the bean as homotypic conditioning and the conditioning of the bean by another species as heterotypic conditioning. The mean number of eggs laid by groups of three females showed that *C. maculatus* had a significantly higher ($P<0.001$) oviposition rate than *C. rhodesianus* in all treatments (Table 1). The

number of eggs laid by *C. rhodesianus* is decreased significantly only by heterotypic conditioning while oviposition of *C. maculatus* was significantly reduced by both types of conditioning. *C. rhodesianus* laid more than twice the number of eggs on clean or homotypically conditioned beans than in beans conditioned by the other species (Table 1). Although oviposition of *C. maculatus* was affected by homotypic and heterotypic conditioning, the proportional reduction in the number of eggs laid was less than *C. rhodesianus*. The inhibition of egglaying of *C. rhodesianus* by *C. maculatus* could be an important factor in determining the outcome of competition between the two species (GIGA, 1982). Similar work by YOSHIDA (1961, 1966) indicated that the fecundities of *C. chinensis* and *C. maculatus* were not affected by conditioning, but Yoshida found that hatching of *C. maculatus* eggs was decreased by both homotypic and heterotypic conditioning while *C. chinensis* was not affected. In this study, no statistically significant effect of conditioning on the hatchability of eggs of either species was found (Table 2). The hatchability of *C. rhodesianus* was significantly ($P<0.001$) less than that of *C. maculatus*.

Oviposition on beans conditioned by virgin adults : The analyses of variance of the square root transformation of the total number of eggs showed that both species liad fewer eggs on conditioned grain compared with clean grain (Tables 3 and 4). Oviposition of *C. rhodesianus* was not significantly affected by homotypic conditioning by either sex. However, egg-laying of *C. rhodesianus* was significantly reduced on beans conditioned by either sex of *C. maculatus* (Table 3). Oviposition of *C. maculatus* (Table 4) was not influenced by heterotypic conditioning or by *C. maculatus* females. However, significantly fewer eggs were laid by *C. maculatus* females on grain conditioned by *C. maculatus* males. The results indicate that egg-laying of *C. rhodesianus* is more affected by conditioning by *C. maculatus* than by homotypic conditioning, while *C. maculatus* is affected more by homotypic conditioning than by *C. rhodesianus*. YOSHIDA (1966) found that *C. chinensis* and *C. maculatus*, when given a choice, deposited their eggs on clean beans and avoided beans conditioned by virgins of either sex; both species laid more eggs on the beans conditioned by either sex of *C. maculatus* than on beans conditioned by *C. chinensis*. However, fewer eggs were

TABLE 1. Mean number of eggs (square root transformation) laid on beans conditioned by males (back transformation in parenthesis).

	C. maculatus	*C. rhodesianus*
Conditioned by *C. maculatus*	12.5 (156.3)	5.1 (26.0)
Conditioned by *C. rhodesianus*	13.0 (169.0)	7.5 (56.3)
Clean beans (Control)	14.2 (201.6)	7.7 (59.3)
Mean	13.2 (174.2)	6.8 (46.2)

Standard error of difference between any two tansformed means in body of table = 0.8.

TABLE 2. Percentage (angular transformation) eggs hatched on beans conditioned by males (back transformed means in parenthesis).

	C. maculatus	*C. rhodesianus*
Conditioned by *C. maculatus*	72.9 (91.3)	13.9 (5.8)
Conditioned by *C. rhodesianus*	73.2 (91.6)	38.5 (38.7)
Clean beans (Control)	79.4 (96.6)	28.3 (22.5)
Mean	75.2 (93.5)	26.9 (20.5)

Standard error of the difference between species means = 5.2.

TABLE 3. Mean number eggs laid by *C. rhodesianus* on bean conditioned by virgin adults.

		Number of eggs	
Conditioning reatment		Square root transformation	Back transformation
C. maculatus	Males	4.0	16.0
	Females	4.4	19.4
C. rhodesianus	Males	5.4	29.2
	Females	5.6	31.4
Control		5.5	30.2

Standard error of the difference between any two transformed means = 0.6.

TABLE 4. Mean number of eggs laid by *C. maculatus* on beans conditioned by virgin adults.

		Number of eggs	
Conditioning treatment		Square root transformation	Back transformation
C. maculatus	Males	7.0	49.0
	Females	7.7	59.3
C. rhodesianus	Males	8.0	64.0
	Females	7.5	56.3
Control		7.9	62.4

Standard error of the difference between any two transformed means = 0.3.

laid when beans were conditioned by females rather than males. MESSINA & RENWICK's (1985) results confirmed that the passage of virgin females of *C. maculatus* had no deterrent effect and males were actually attractive, resulting in seeds over which they had walked being preferred to the controls for oviposition. Their results imply that adults of both sexes may produce biological conditioning substances having similar effects on oviposition but different levels of inhibiting influence. In *Acanthoscelides obtectus* (Say) SZENTESI (1981) suggested that the substances were not necessarily the same for males and females and that the chemicals involved were used for "oviposition-deterring purposes" only. The level of oviposition inhibition by substaces deposited either by mated or virgin females of *A. obtectus* was not as high as in the case of beans marked by males (SZENTESI, 1981). In contrast, OSHIMA *et al.* (1973) found greater inhibitory activity with extracts from beans conditioned by females of *C. chinensis* than from beans conditioned by males. OSHIMA *et al.* (1973), YAMAMOTO (1976) and HONDA *et al.* (1976) all found that both males and females of *C. chinensis* deposited substances which were a complex mixture of hydrocarbons, fatty acids and triglycerides. None of these authors separated female products associated with eggs from any others which they may deposit at other times (eg. during creeping, or exeretion on beans). The literature suggests that male and female bruchids both produce oviposition deterrents, the latter in association with eggs. CREDLAND & WRIGHT (1990) attempted to distinguish between the effects of materials deposited by the two sexes of *C. maculatus* and the effects of compounds produced by females in association with eggs and any others produced during other activities. They emphasized that the eggs themselves may not be the site of the product produced by females but that the physical presence of the eggs cannot be overlooked in any discussion of the possible role of pheromones in oviposition and egg distribution by bruchids. The results of the present study demonstrates that both sexes of *C. maculatus* and *C. rhodesianus* adults produce compounds which have major effects on oviposition. There is some evidence that different *Callosobruchus* species may be able to recognise and respond differently to each other's products.

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VARIATION IN HAEMOCYTE TYPES WITH REFERENCE TO REPRODUCTIVE ACTIVITY IN *BLATTELLA GERMANICA* L. (DICTYOPTERA: BLATTELLIDAE) AND THE OCCURRENCE OF UNDESCRIBED HAEMOCYTE TYPES IN SOME ADULT STAGES

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A total of 10 different types of haemocytes have been recorded from the haemolymph of adults of *Blattella germanica*. Podocytes and vermicytes, not noticed by earlier workers, are reported. A new type of haemocyte (with characteristics not described thus far in any insect) has been noticed in mature adults and is designated as Spinocyte (SN). The morphology and morphometric variations of various haemocytes in relation to reproductive activity have been described and descussed.

(*Key words:* haemocytes, reproductive activity, *Blattella germanica*)

INTRODUCTION

Haemocytes and variation in the population of various types have been studied in the German cockroach, *Blattella germanica* mainly with reference to development (HAZARIKA & GUPTA, 1987) or immune reaction to foreign tissues (HAN & GUPTA, 1988). A specific defence process like encapsulation of implanted material has also been described (HAN & GUPTA, 1989). However, the haemocytes and variation in the populations of various types with reference to reproduction, a normal function of adult insect, have not attracted the attention of any worker so far. The morphology and morphometric variations of various haemocyte types are persented in this paper.

MATERIAL AND METHODS

Adults of both sexes of *B. germanica* were obtained from a laboratory colony of insects maintained at temperature ranging from 26°–30°C and reared on bread crumbs and water, provided *ad libitum*.

Haemolymph samples of the following stages were studied for haemocytes: (a) Sexually immature adults (i.e., less than 4 days old) of both sexes, usually within 4 h of imaginal moult. (b) Mature adults (i.e., age 5 days or more) of both sexes, while still copulating. (c) Mature adults of both sexes, immediately after the insects separated following coition. (d) Female insects carrying oothecae. (e) Female insects, immediately after the deposition of the oothecae. For obtaining the above stages, sexual pairings of insects of the same age were carried out. Insects of a pair were not separated while taking the haemolymph samples of 'copulating' stage. Post-coital females (all of the same age) carrying oothecae were kept in separate jars. Haemolymph samples were taken from cut antennae/femurs/wing bases or after puncturing the abdomen. Preliminary identification of haemocytes was carried out from the hanging drop preparation of fixed haemolymph using the identification key given by GUPTA (1979a). Heat fixed

and air dried blood films were prepared and stained with Giemsa as per ARNOLD & HINKS (1979). Giemsa staining has been considered to be the best by SHAPIRO (1979). Observations were made using light and phase contrast microscope (Leitz-Dialux 20). Measurements were done on haemocytes in these preparations using ocular and stage micrometers.

RESULTS AND DISCUSSION

Ten types of haemocytes were distinguished from the fixed heamolymph preparations of various stages of *Blattella germanica* (Fig. 1.) using the classification of JONES (1962) and GUPTA (1979 a, b, c). These ten types are prohaemocytes (PR). plasmatocytes (PL), granuloctyes (GR), spherulocytes (SP), adipohaemocytes (AD), oenocytoids (OE), coagulocytes (CO), podocytes (PO), vermicytes (VE) and a type unlike any other described thus far with specific characteristics, which has been tentatively named spinocytes (SN). All these haemocyte types could easily be distinguished under light/phase contrast microscopy. The cytological characteristics and morphometry of the haemocytes are presented in Tables 1 and 2 respectively. CHIANG *et al.* (1988) had recognised only seven types of haemocytes in the same insect, the types not reported being podocytes (PO), vermicytes (VE) and spinocytes (SN). Of these, POs and VEs were not recognised as distinct types in electron microscopic studies by DEVAUCHELLE (1971). CHIANG *et al.* (1988) did not see them with light microscope also. It is notable that of the ten types, six (PL, GR, SP, AD, OE and CO) have been found to be present in all the stages studied (Table 2). Prohaemocytes (PR) were present in the freshly moulted (sexually immature) adults of both sexes and in females carrying oothecae, but absent in copulating/post coital adults and females after deposition of oothecae. Podocytes (PO), on the other hand, were present in the haemolymph of adults of all stages except sexually immature adults of both sexes and females carrying oothecae. Similarly, spinocytes (SN) were noted in all adult stages except the sexually immature ones. Vermicytes (VE) were noted only in the haemolymph of a copulating female.

Some major trends with regard to the morphology and morphometry of these haemocytes are as under.

(i) Prohaemocytes (PR) were generally seen as large, single cells in the sexualy immature adults while they were seen as small cells bunched together in females carrying oothecae. This suggests a recent release from the haemopoietic tissue and a possible role at this stage. One can only conjecture about the possible function which may have some thing to do with the needs of the developing nymphs. Various earlier workers like YEAGER (1945) and BEAULATON & MONPEYSSIN (1977) had suggested that PRs got transformed to other haemocyte types as per requirements

Photographs of various haemocytes of adult *Blattella germanica*: 1. Prohaemocytes (PR) × 1000; 2, 3. Plasmatocytes (PL) × 400. Difference in shape is noticeable in the two PLs. Protoplasmic extension is visible in 3 (arrow). 4, 5. Granulocytes (GR) × 400. 6. A spherulocyte (SP) × 1000. 7. An adipohaemocyte (AD) × 400. 8. An Oenocytoid (OE) × 400. 9, 10. Coagulocytes (CO). Difference in shape is noticeable in the two COs. 9×10000, 10×400. 11. A Podocyte (PO), Long protoplasmic extensions are noticeable. × 400. 12. A vermicyte (VE)× 1000. 13, 14. Spinocytes (SN) which appear to be transformation of PRs. Both PRs and SNs are visible in 13 (× 1000). Microphotograph 14 shows a number of SN. Clear cytoplasm, large nucleus and spiny cell membrane can be noticed in a number of these cells (arrows) × 1000. Microphotographs 2, 3, 4, 5, 6, 7, 10, 12, and 14 were taken under phase optics, others Giemsa staining.)

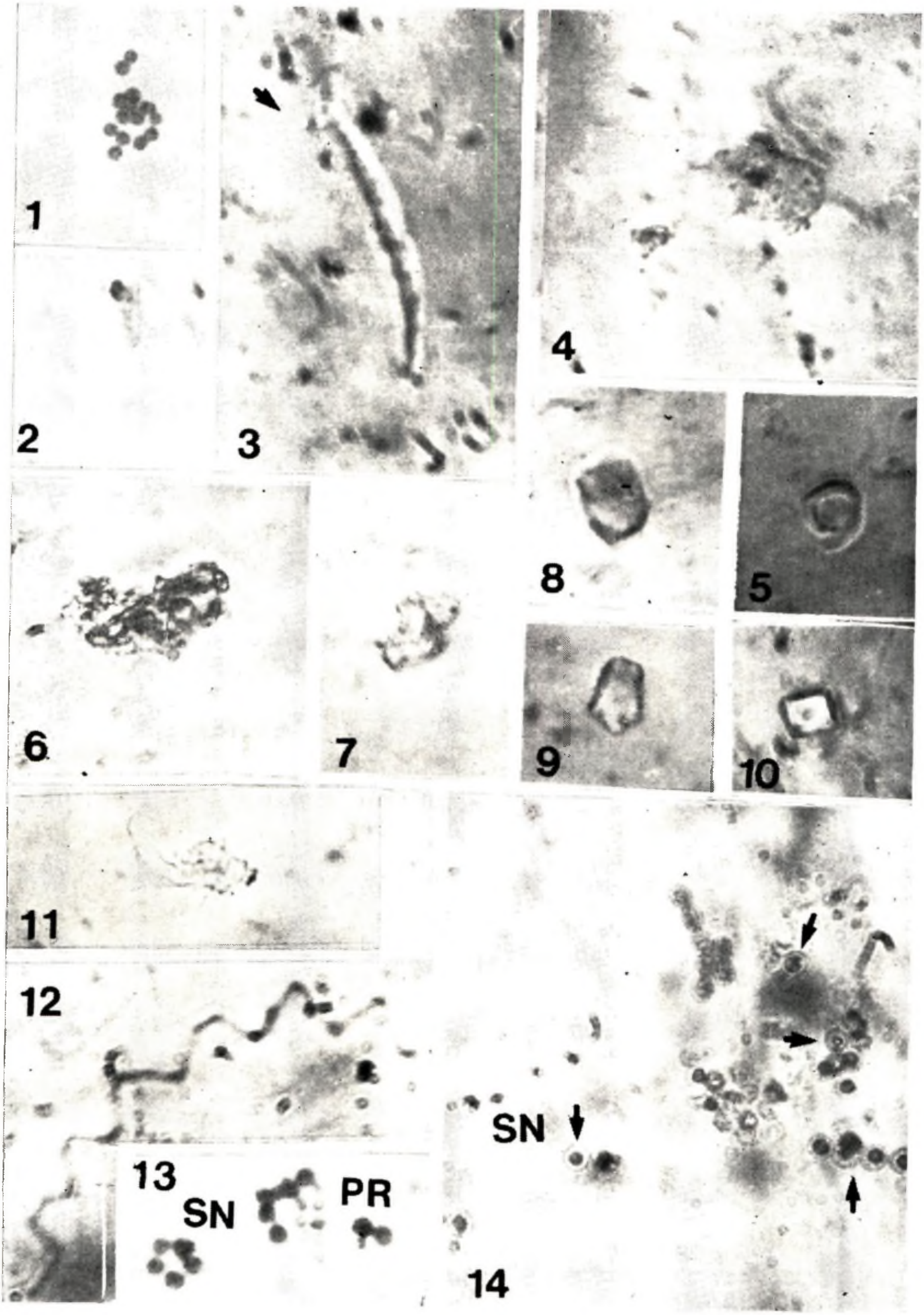
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TABLE 1. Cytological characteristics of haemocytes of the adult *Blattella germanica*.

Haemocyte Type	Shape	Cytoplasm	Nucleus	Other characteristics
Prohaemocyte (PR)	Small, round/ovoid; cell outline: smooth	Perinuclear space very small, cytoplasm: dense, homogeneous	Large, round, centrally located	Cells mostly found in bunches
Plasmatocyte (PL)	Small to large, round/ovoid/fusiform/irregular; cell outline: smooth/irregular	Granular/agranular	Uninucleate/binucleate; nucleus small, round, ovoid, centrally/eccentrically located.	—
Granulocyte (GR)	Round/ovoid/irregular; cell outline: mostly irregular	Granular	Small, round, centrally located	—
Spherulocyte (SP)	Small to large, irregular; cell outline:irregular	Contains membrane-bound intracytoplamic spherules of varying size	Round/ovoid, centrally located	—
Adipohaemocyte (AD)	Round/oval;cell outline, smooth	Contains small, round refringent fat droplets	Round/ovoid; eccentrically located	—
Oenocytoid (OE)	Large; irregular in shape; cell outline: smooth or irregular	Granular/agranular, contains plate/rod like inclusions	Round/elongated; centrally/eccentrically located	—
Coagulocyte (CO)	Small to large squarish/rhomboidal; cell outline: sharp	Hyaline with some granular inclusions	Small, round/ovoid; centrally/eccentrically located	—
Podocyte (PO)	Small to very large with several cytoplasmic extensions; cell outline irregular due to the presence of several micropapillae and filopodia	Arganular	Large, round/lobate, centrally located	In smaller cells the cytoplasmic extensions anastomose
Vermicyte (VE)	Extremely elongated; cell outline: smooth	Agranular	Round, eccentrically located	—
Spinocyte (SN)	Small/round/crescent like; cell outline: spiny	Perinuclear space small, granular with concentric rings	Large, round, centrally located	Present singly or in groups or strings

TABLE 2. Variation in the mean size of haemocytes (in μm) in different reproductive stages of *B. germanica*. All values are mean of three readings.

Haemocyte types	Stage							
	Immature adults		copulating		post-coital		Females carrying oothecae	Female after deposition of oothecae
	Male	Female	Male	Female	Male	Female		
Prohaemocytes (PR)	5.8x4.8	10.5x7.3	—	—	—	—	3.0x3.2	—
Plasmatocytes (PL)	13.2x9.8	14.1x11.9	13.2x8.1	7.1x4.7	11.7x6.7	11.0x5.8	11.8x6.1	11.8x7.3
Granulocytes (GR)	11.9x9.7	14.1x11.9	12.7x8.7	7.3x6.3	8.0x7.3	15.4x13.1	8.5x8.5	8.5x7.5
Spherulocytes (SP)	17.6x12.8	20.6x9.7	20.0x12.2	15.2x9.5	23.7x15.0	20.4x14.0	13.6x7.3	16.3x9.6
Adipohaemocytes (AD)	15.1x13.6	17.2x15.1	12.9x10.5	12.0x8.8	10.7x10.7	14.2x14.2	17.4x12.7	13.0x12.7
Oenocytoids (OE)	24.2x14.5	22.8x14.6	18.7x11.2	16.7x9.2	17.4x8.0	20.3x12.9	26.8x19.0	8.5x7.3
Coagulocytes (CO)	20.4x13.2	22.3x12.4	13.0x9.9	8.2x5.4	15.1x9.7	12.1x7.0	12.4x7.3	18.8x9.4
Podocytes (PO)	—	—	72.3x38.7	40.6x33.7	8.8x8.8	36.5x29.2	—	40.2x25.2
Vermicytes (VE)	—	—	—	58.4x1.5	—	—	—	—
Spinocytes (SN)	—	—	4.1x4.1	3.7x3.7	3.8x3.8	5.1x3.7	4.4x4.4	3.7x3.7

of the insect. But the present observation suggests some definite, additional, post-ecdysical function for these cells in the adult stages of *B. germanica*.

(ii) Plasmatocytes (PL) and granulocytes (GR) have been referred as *immunocytes* by GUPTA (1985a, 1986) as these cells are primarily responsible for immunological functions like phagocytosis and encapsulation of foreign antigens. Secretion of haemagglutinins, antibacterial and antiviral factors, complement like factors, coagulation and wound healing are some of the other functions attributed to GRs (GUPTA, 1979c, 1985a, 1986). SINGH *et al.* (1991) have also reported phagocytic function of GRs against a fungus *Curvularia lunata* in the heteropteran *Nezara viridula*. HAN & GUPTA (1988) have further concluded that GRs provide both phenols and phenoloxidase that bring about melanization during encapsulation. Furthermore, GUPTA (1986) had compared GRs of arthropods with B- and T- lymphocytes of vertebrates. In view of these functions of PLs and GRs,

it is interesting to note the size variations of these cells during the adult stags. PLs show a decrease in size from sexually immature adults to post-coital adults. GRs however, show size variation related to sex; these cells being largest in post-coital females and smallest in males of the same stage. Whether the cellular defence requirements of the female are higher and whether GRs are more involved in this process, are questions which remain to be answered.

(iii) The Spherulocytes (SP) are smallest in the females carrying oothecae, followed by females after deposition of oothecae.

(iv) Adipohaemocytes (AD) and Oenocytoids (OE) did not show size variations of any significance during the various adult stages although OEs were found to be very small in females after deposition of oothecae.

(v) Coagulocytes (CO) were the largest in sexually immature adults but smallest in mating adults. Ths size variation may be related to the difference in coagulative capability and phenoloxidase carrying capacity. These cells may be cartwheal like in appearance or may appear irregular in shape.

(vi) Podocytes (PO) showed wide variation in size, being very large in mating males and very small in post-coital males. The size remains more or less unchanged in females of various stages. These cells were not noticed in sexually immature adults and females carrying oothecae. When the cells were small, their cytoplasmic extensions anastomosed forming a network of many cells. The significance of the formation of this network is not known.

(vii) In all adults except the immature ones, were seen cells with structural peculiarities unlike any other cells described so far. These cells are small, round, oval or cresentic in shape, each with a large nuclues. They appeared like PRs but had the cell membrane that appeared more distict and spiny. These cells have therefore, been tentatively termed Spinocytes (SN). Like PRs these cells are found singly, in strings or hollow hexagons.

It may be added that the crescent cells described in the cockroach *Gromphadorhina portentosa*, described by RITTER (1926) and later identified as Oenocytoids by GUPTA (1985 b) were not noticed in *Blattella*.

Vermicytes were recognised in the haemolymph of *Prodenia* by YEAGER (1945) who called them *vermiform cells*. TUZET & MAINER (1959) described them as '*giant fusiform cells*'. COSTIN (1975) noted such cells in the haemolymph of *Periplaneta americana* and GUPTA (1968, 1979b, c) has preferred the term *vermicyte*. Arnold (1974) stated that they occur mainly just prior to pupation but never in large numbers. VEs have however, been seen in copulating female adult in the present observation.

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INFLUENCE OF JUVENILE HORMONE ANALOGUE ZR-777 ON THE ENDOCRINES OF THE FIFTH INSTAR NYMPHS OF *DYSDERCUS CINGULATUS* FABR. (HETEROPTERA: PYRRHOCORIDAE)[1]

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The endocrine system of the fifth instar nymphs of *Dysdercus cingulatus* is comparable to that of the adult. Size of the cells and the nuclei and the volume of glands as well as uptake of labelled glycine and thymidine have been taken as criteria for synthetic activity of endocrine glands. All endocrine glands start with comparatively low activity in the beginning of the fifth instar. Neurosecretory A-cells and corpus allatum show only a single activity cycle, whereas prothoracic gland exhibits two peaks of activity. Application of juvenile hormone analogue (JHA) ZR-777 resulted in supernumerary nymphs with a reduction of 2 days in nymphal life span. Juvenile hormone analogue ZR-777 has an inhibitory effect on A-cell activity. A negative feed back effect is observed on corpus allatum but still corpus allatum remains active. ZR-777 is found to stimulate prothoracic gland cells. Two peaks of prothoracic gland activity observed in normal and control insects merge into a single peak which is sustained for a greater part of the nymphal period in the JHA treated insects. It appears that higher activity of PG cell produces more ecdysone, and more juvenile hormone already present in haemolymph as a result of topical application may be inhibiting NSC. As a result, instead of normal adult, supernumerary insects arise.

(*Key words:* median neurosecretory cells, ccrpus allatum, corpus cardiacum, prothorcic gland, juvenile hormone, ecdysone, morphometry, radioautography)

INTRODUCTION

Inter-relationship among the various endocrine glands of insects, icluding feed-back mechanism in relation to physiological changes have been worked out in many insects. Effect of juvenile hormone on the prothoracic gland and ecdysone has been studied by HIRUMA *et al.* (1978) and SHAAYA *et al.* (1986). Neurosecretory control over the prothoracic gland has been reported by MALA *et al.* (1977), HIRUMA *et al.* (1978) and WATSON *et al.* (1987). Feed-back action of ecdysone and juvenile hormone has also been studied by SIEW & GILBERT (1971), KORT & GRANGER (1981) and EDWARDS *et al.* (1987).

However a comprehensive study involving interaction of hormones produced by these endocrine glands in a single animal appeared to be lacking and hence it was thought worthwhile to study the interaction of endocrine glands during last nymphal instar (5th instar) and during moulting in the red cotton bug. The results formed

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the thesis of the author (JOSEPH, 1981) and are reported in the present paper.

MATERIALS AND METHODS

The last (5th) instar nymphs of *Dysdercus cingulatus* required for this work were raised in the laboratory on soaked cotton seeds.

Neuroendocrine complex consisting of the brain, corpus cardiacum (CC) and corpus allatum (CA) of the zero day to six day old fifth instar nymphs at 24 hour interval, and of newly moulted adult males and females, were dissected out and fixed in BOUIN'S fixative. Brains were either stained whole using paralydehyde fuchsin (CAMERON & STEELE, 1959) or resorcin fuchsin (ITTYCHERIA & MARKS, 1971). Sections at 5 μm thickness were stained using Gomori's chrome alum haematoxylin phloxin (GOMORI, 1941) and in Heidenhain's iron alum haematoxylin and eosin.

The quantity of neurosecretory material (NSM) present in the neurosecretory cells (NSC) of the pars intercerebralis of the brain was represented as neurosecretory index from the whole mount preparations of the brain (KRISHNANANDAM & RAMAMURTY, 1971). The size (maximum length and breadth) of the neurosecretory cells and their nuclei was also measured.

The area of freshly dissected corpus allatum was calculated from camera lucida drawing essentially after the method followed by SCHARRER & VON HARNACK (1958). The diameter of the nuclei of the corpus allatum was measured using calibrated ocular micrometer.

The prothoracic glands (PG) were studied under phase contrast microscope. The volume of the cell was calculated using the formula $4/3\ \pi\ r^3$ or $4/3\ \pi\ ab^2$ (PENZLIN, 1971) depending upon whether the cells were spherical or oval.

Application of Juvenile Hormone Analogue (JHA): Kinoprene (ZR–777) from Zoecon corporation, was the juvenile hormone analogue employed for the present study. Kinoprene was dissolved in acetone so as to get a concentration of 1 μg/μl and was topically applied on insects with the help of Hamilton microlitre syringe. From pilot experiments topical application of ZR–777 at the tip of the abdominal region, was found to be most effective and the minimum effective dose required for 50% fifth instar nymphs to moult into sixth instar nymphs was found to be 0.25 μg. ZR–777 (0.25 μg) was applied to the newly moulted fifth instar nymphs. First lot of the H^3 JHA treated and acetone-treated control insects were sacrificed at fourth hour after treatment and thereafter at 24 h intervals upto subsequent moulting. Neurosecretory cells, corpus allatum and prothoracic glands of experimental and control insects were studied as described previously.

Radioautography:– Radioautography was carried out after injecting tritiated glycine (glycine-2-T, specific activity: 840 mCi/mM) and tritiated thymidine (Thymidine-methyl–T, specific activity; 6500 mCi/mM). (from Department of Atomic Energy, Trombay, Bombay, India). Tritiated material was diluted so as to get 0.5 μCi/1 μl of solution. Zero day old fifth instar nymph weighing 20 mg was injected with 0.5 μCi of tritiated material. The dosage of tritiated material was increased by 0.5 μCi for every 10 mg increase of body weight and for older insects original tritiated material (1 μCi activity per μl solution) was made use of to avoid too much volume to be delivered. Table 1 indicates the sets of experiments conducted.

TABLE 1. Experimental schedule of injection of tritiated glycine and tritiated thymidine in to ZR-777 treated and control insects.

Experiment	Control I	Control II (cold)	Age of the insect (days)	Quantity of labelled material injected (μ Ci)	Time of sacrifice after injection
ZR-777 applied, H^3 glycine injected	Acetone applied H^3 glycine injected	Acetone applied glass distilled water injected	0 day 1 day 2 day 3 day 4 day 5 day (moulted) 6 day (moultd)	0.5 0.75 1.0 1.5 2.0 2.5 3.0	2 h, 4 h, 6 h, 8 h, and 16 h, (this time schedule repeated for insects of all ages)
ZR-777 applied, H^3 thymidine injected	Acetone applied H^3 thymidine injected	Acetone applied glass distilled water injected	0 day 1 day 2 day 3 day 4 day 5 day (moulted) 6 ay (moulted)	0.5 0.75 1.0 1.5 2.0 2.5 3.0	2 h, 4 h, 6 h, 8 h, and 16 h. (this time schedule repeated for insects of all ages)

After anaesthetizing insects with ether, injections were given with a Hamilton microlitre syringe. Insects that showed haemolymph loss were discarded. Injected insects were kept in chimneys and allowed to recover from anaesthesia. They were fed on soaked cotton seeds. Each day, groups of five insects were sacrified at 2 h, 4 h, 6 h, 8 h and 16 h after injection of labelled material. The brain together with corpus allatum and prothoracic glands were dissected out and fixed in Bouin's fluid. The fixed tissues were processed and paraffin sections were cut at 4 μm thickness, as already described.

The sections were deparaffinised and dipped in Kodak NTB-2 liquid emulsion (PRESCOTT, 1964) diluted 1:1 with water. Dried slides were stored in the dark at 4° C. They were developed in Kodak D-19 developer, after optimum exposure, which was found to be 7 days for tissues with tritiated thymidine and 11 days for tissues with tritiated glycine. The slides were stained with toluidine blue (BOYENVAL & FISCHER, 1976) cleared and mountd in DPX. All the control slides were also processed in the same way.

Grains were counted with the aid of an ocular grid divided into squares of 2 μm. Counts from 10 A-type neurosecretory cells from 5 different insects, as well as prothoracic glands and corpus allatum were taken for evaluation. The counts of silver grain over the ordinary neurons were subtracted to find out the differential

incorporation of labelled material into A–cells, corpus allatum and prothoracic glands.

Silver grains on cold control sections were also counted to find out background radiation, which was found to be negligible.

When the same exposure time was given for all the experimental tissues, it was observed that grains were grouped in some of them due to very high incorporation. In cases where the grains were grouped and could not be individually counted, '+' sign was given to denote a mild clumping and '++' sign for thick clumping, and the data presented accordingly.

OBSERVATION

General Structure of the Neuroendocrine System:- The endocrine organs of the fifth instar nymphs of *Dysdercus cingulatus* consist of the neurosecretory cells of the brain, corpus cardiacum, corpus allatum and prothoracic glands. Based on the methods employed, neurosecretory cells could be detected only in the pars intercerebralis, close to the median line. Depending on size, position and stainablity three neurosecretory cell types are observed. They are A, B and C (Figs. 1, 2). The position, number, shape, size and staining characteristics of the cell and the nuclei are summarised in Table 2.

Axons from the A–NSC converge towards the centre of the pars intercerebralis, cross over to the opposite sides and extend backwards as two tracts. They emerge from the brain as nervi corpori cardiacii–I, from either side of the brain and pass behind, entering the aortic wall where they terminate after repeated branching. The axons from B–and C–cells could not be traced during the present study and also no changes have been noticed in the size and stainability of these cells. Neurosecretory granules comparable to those observed in the A–cells in thier stainability are seen in the aorta also (Fig. 3).

Corpora cardiaca (CC) are paired trianguler structures seen just behind the brain. These are attached to the aorta laterally. Chromophil cells measuring 4.4 μm to 6.6 μm in diameter and chromophobe cells measuring 2.2 μm to 3.3 μm in diameter are observed in the corpus cardiacum. The axon endings of the NSC are not seen in corpus cardiacum (Fig. 4).

Corpus allatum (CA) is usually an unpaired gland rather globular but slightly flattened dorsoventrally. It is seen just behind the CC and ventrolaterally to the aorta to which it is attached anteriorly. Neurosecretory axons have not been observed in CA. Cells are closely packed. Cell boundaries and nuclei can be clearly distinguished (Fig. 4).

Prothoracic glands (PG) are seen concealed beneath the thoracic fat body, diffusely arranged among the tracheae. The glands extend between the CA and the posterior end of the salivary gland of that side. Each gland consists of about 100 to 200 cells held loosely together by intercellular fibrils. The PG cells are spherical or elliptical in shape. Cytoplasmic vacuoles and granules are observed in the PG cells under phase contrast microscope. In addition, two zones of cytoplasm are distinguishable in the living cells; an inner lighter and peripheral thin darker region (Figs. 5, 6).

Axons from the neurosecratory A-cells have been traced into the aorta. After entering the aorta the axons ramify at the anterior region. Neurosecretory granules comparable to those observed in the A–cells in their stainability are seen in the aorta.

Fig. 1. Section of the brain showing A-cells (A-NSC) and B-cells (B-NSC) (chrome alum haematoxylin phloxin) × 400.

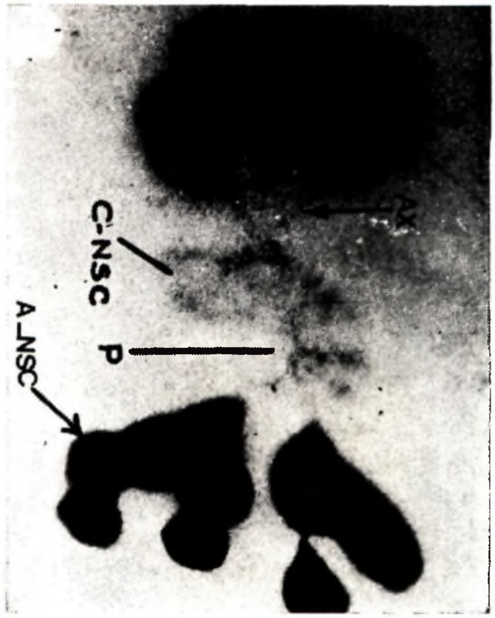

Fig. 2. Whole mount of brain of fifth instar nymph showing A-cells (A-NSC), C-cells (C-NSC), axons from neurosecretory cells (AX) point of convergence of axons from different NSC (P). (Paraldehyde fuchin) × 400.

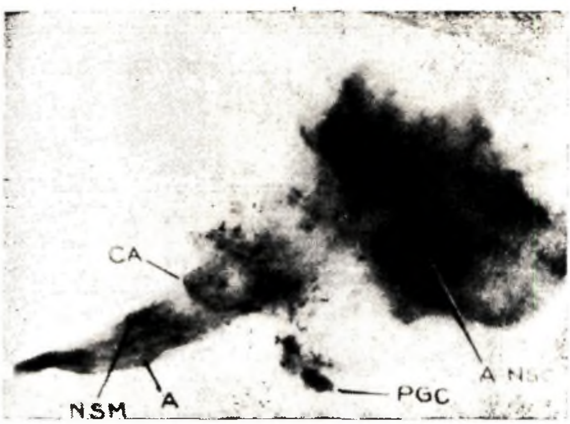

Fig. 3. Whole mount of endocrine system with anterior portion of aorta (A) showing neurosecretory material (NSM) from the axon fibers of A-cells (A-NSC), corpus allatum (CA) and prothoracic gland ell (PGC) × 10.

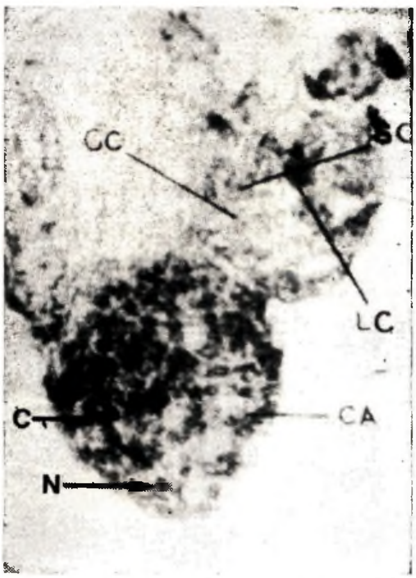

Fig. 4. Section of corpus allatum (CA) and corpus cardiacum (CC) showing larger chromophil cells (LC) smaller chromophil cells (SC) and corpus allatum with closely packed cells (C) and nucleus (N) × 400.

Fig. 5. Prothoracic gland cells round showing nucleus (N), inner thinner cytoplasm (TC) and outer thicker cytoplasm (OTC) × 1000.

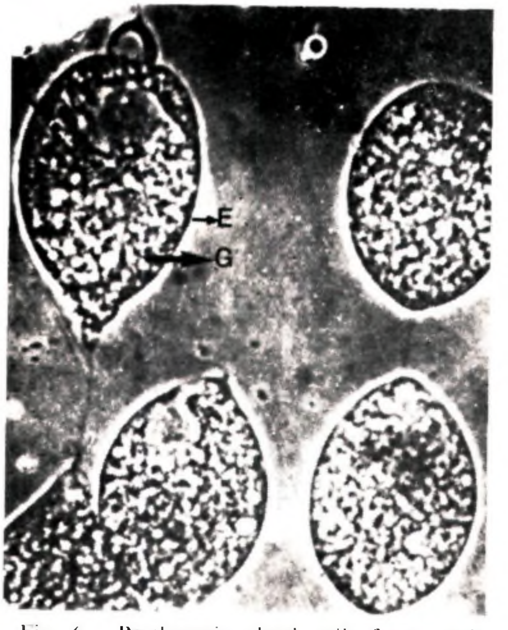

Fig. 6. Prothoracic gland cells from adult insect showing elliptical translucent cells (E) with granules (G) × 1000.

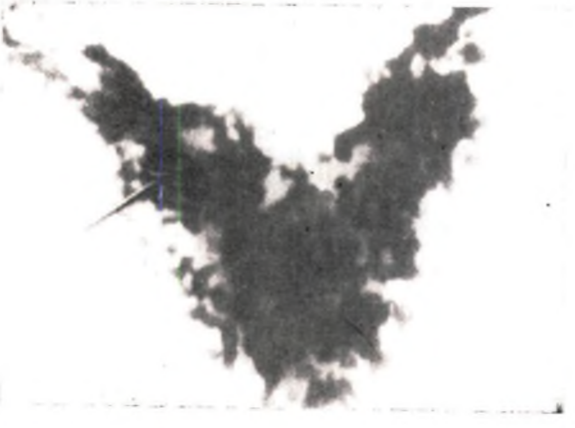

Fig. 7. A-cells of ZR-777 (0.25 μg) treated insect (on zero day). 1.0 μCi of H^3 glycine was injected into two day old insect. Sections were taken at fourth hour of second day. Showing incorporation of H^3 glycine × 1000.

TABLE 2. The characteristics of the different types of neurosecretory cells present in the pars-intercerebrails of brain of fifth instar nymphs of *Dysdercus cingulatus*.

Type of cells	Position of cells	Shape of cells	Number of cells	Staining reaction					Cell size (Mean of length and breadth) in μm		Nuclear size (Mean of length and breadth) in μm	
				PF	RF	PAVB	CHP	ABP	Male	Female	Male	Female
A	Seen as two groups on either side of the median furrow of the brain	Spherical/ pear shaped	18	Deep purple	Deep purple	Greenish blue	Blue black	Turqu-oise	17.1	17.9	5.3	5.8
B	Scattered around and among A-cells	Spherical	8–13	Not stain-able	Not stain-able	Not stain-able	Red	Red	10.0	10.0	4.0	4.0
C	Between the two groups of A-cells	Elliptical	4	Faint purple	Faint purple	Not stain-able	Not stain-able	Not stain-able	13.0	13.0	3.5	3.5

PF – Paralydehyde fuchsin; RF – Resorcin fuchsin; PAVB – Performic acid victoria blue; CHP – Chrome alum haematoxylin phloxin; ABP – Alcian blue phloxin.

Hence the aorta wall apparently functions as a neurohaemal organ (NHO) (Fig.3).

Changes in the neuroendocrine system during fifth instar nymphal period:- Remarkable changes have been noticed in the neurosecretory A–cells, CA and PG cells of the fifth instar nymphs during the developmental period whereas such changes are not conspicuous in B–cells, C–cells and CC. Axon endings of the A–cells in the wall of the aorta (NHO) contain comparatively little secretory material at any time when compared to the total quantity of material in the A–cell perikaryon of the pars intercerebralis. Hence, the data on B–cells, C–cells, CC and of aorta are not reported here.

Changes in the A- cells:- During the six days of fifth instar nymphal span neurosecretory material in the A–cells, cell size and the size of their nuclei show striking quantitative fluctuations. Both in males and females, the estimated neurosecretory index rises upto fifth and thereafter it decreases till moulting (Table 3). The cell size and nuclear size initially low, gradually increases. Cell size is greatest on the fourth day whereas nuclear size is maximum on third day. In females the cell size is slightly greater than that in the males. However, both follow the same changing pattern throughout the nymphal period. (Table 4.)

Changes in CA:- Data on changes in the CA are given in the Table IV. It may be noticed that the volume of the gland and that of the nucleus gradually increases both in male and female, reaching maximum on the fifth day. Subsequently the volume decreases. The differences between males and females are found to be not significant. CA cell nuclear size is also maximum on fifth day and then it decreases (Table 5).

TABLE 3. Changes in the neurosecretory material of A–cells of fifth instar numphs during development (Mean of 20 observations).

Age of fifth instar nymphs (days after emergence)	Number of A-cells grouped as per their secretory content						Index of NSM = Sum of the product of number of cells and secretory contents		t values
	Male+	Female	Male+ +	Female	Male+ + +	Female	Male	Female	
Zero day	14	13	4	5	—	—	22±0.65	23±0.68	1.055
One day	10	9	8	8	—	1	26±1.10	28±1.23	1.212
Two day	2	1	16	15	—	2	34±1.71	37±0.54	1.673
Three day	1	—	13	15	4	3	39±0.89	39±0.85	0.000
Four day	—	—	10	6	8	12	44±0.76	48±0.89	3.418**
Five day	—	—	3	2	15	16	51±0.59	52±0.73	1.065
Six day	—	—	12	11	6	7	42±0.84	43±0.89	0.817
Normal adult	9	9	7	6	2	3	29±1.70	30±1.60	0.428

** Significant at both 5% and 1% level.

TABLE 4. Changes in cell and nuclear size of A-cells of fifth instar nymphs during development (Mean of 20 observations).

Age of fifth instar nymphs (days after emergence)	A-Cell size in μm		t value	A-Cell nuclear size in μm		t value
	Male	Female		Male	Female	
Zero day	17.1 ± 0.46	17.9 ± 0.62	1.036	5.3 ± 0.80	5.8 ± 0.34	0.575
One day	17.9 ± 0.63	18.8 ± 0.49	1.128	6.1 ± 0.31	6.4 ± 0.35	0.642
Two day	18.7 ± 0.49	91.3 ± 0.51	0.848	6.5 ± 0.33	7.3 ± 0.32	1.740
Three day	23.1 ± 0.49	23.6 ± 0.46	0.744	10.9 ± 0.33	11.5 ± 0.33	1.286
Four day	23.6 ± 0.51	26.8 ± 0.49	4.525**	8.9 ± 0.33	9.6 ± 0.42	1.310
Five day	22.7 ± 0.46	26.2 ± 0.50	5.151**	7.5 ± 0.43	7.3 ± 0.29	0.386
Six day	21.2 ± 0.48	22.2 ± 0.42	1.567	6.2 ± 0.37	6.4 ± 0.37	0.382
Normal adult	19.8 ± 0.53	19.8 ± 0.57	0.000	4.9 ± 0.29	5.2 ± 0.26	0.770

** Significant at both 5% and 1% level.

TABLE 5. Changes in area, volume and nuclear size of corpus allatum of fifth instar nymphs during development (mean of 20 observations).

Age of fifth instar nymphs (days after emergence)	Area of CA in μm^2		Volume of CA in μm^3		t value	Nuclear size of CA cell in μm (mean of diameter)		t value
	Male	Female	Male	Female		Male	Female	
Zero day	277	283	7870 ± 111.3	7760 ± 106.3	0.585	1.7 ± 0.17	1.8 ± 0.17	0.416
One day	293	308	9749 ± 169.2	9851 ± 103.1	0.515	2.0 ± 0.14	2.0 ± 0.16	0.000
Two day	348	369	10949 ± 129.5	11129 ± 142.4	0.936	2.7 ± 0.17	2.8 ± 0.21	0.370
Three day	396	433	16827 ± 143.2	17173 + 139.5	1.731	3.1 ± 0.14	3.2 ± 0.25	0.349
Four day	512	531	24561 ± 151.7	25224 ± 127.3	3.247**	4.0 ± 0.17	4.5 ± 0.17	2.080*
Five day	720	784	32489 ± 109.5	34996 ± 159.8	2.941**	5.3 ± 0.22	5.9 ± 0.29	1.885
Six day	615	638	20007 ± 171.7	20095 ± 137.3	0.400	4.9 ± 0.17	5.2 ± 0.29	0.812
Normal adult	565	592	14781 ± 181.2	15195 ± 108.8	2.786**	3.9 ± 0.17	4.2 ± 0.22	1.079

* Significant at 5% level.

** Significant at both 5% and 1% level.

TABLE 6. Changes in cell and nuclear size of prothoracic gland cells of fifth instar nymphs during development (Mean of 20 observations).

Age of fifth instar nymphs (days after emergence)	Volume of PG cell in μm^3		t value	PG cell nuclear size (Mean of length and breadth in μm)		t value
	Male	Female		Male	Female	
Zero day	25605 ± 1126	29352 ±1341	0.140*	13.4 ± 0.32	13.7 ± 0.29	0.695
One day	62340 ± 1212	62248 ±1512	0.047	15.2 ± 0.31	15.4 ± 0.20	0.542
Two day	81893 ± 1013	82901 ± 982	0.714	25.4 ± 0.72	25.3 ± 0.81	0.092
Three day	67853 ± 920	68452 ± 818	0.478	17.2 ± 0.65	17.1 ± 0.41	0.130
Four day	52301 ± 689	52248 ± 723	0.053	15.7 ± 0.23	15.9 ± 0.35	0.477
Five day	52685 ± 797	52655 ± 1627	0.016	15.8 ± 0.43	15.9 ± 0.41	0.168
Six day	160792 ± 1087	161502 ± 1842	0.332	27.1 ± 0.13	27.4 ± 0.44	0.654
Normal adult	90585 ± 794	91009 ± 864	0.361	20.0 ± 0.71	20.5 ± 0.64	0.523

* Significant at 5% level.

TABLE 7. Changes in the neurosecretory material (NSM) of A-cell of fifth instar nymphs during development. ZR-777 (0.25 μg) was applied on zero day. Control values are given in brackets (Mean of 10 observations).

Age of fifth instar nymphs (days after emergence)	Number of A-cells grouped as per their secretory content			Index of NSM = Sum of the product of number of cells and secretory content		t value
	+	+ +	+ + +			
Zero day (Four h)	4 (13)	12 (5)	2(—)	34.0 ± 0.98	(23 ± 1.07)	7.581**
One day	2 (11)	12 (7)	4 (—)	38.0 ± 0.64	(25 ± 0.68)	13.921**
Two day	1 (5)	13 (12)	4 (1)	39.0 ± 1.29	(32 ± 1.00)	4.129**
Three day	6 (2)	10 (13)	2 (3)	32.0 ± 0.77	(37 ± 1.10)	3.723**
Four day	2 (1)	13 (11)	3 (6)	37.0 ± 1.27	(41 ± 0.39)	3.011**
Five day sixth instar nymph	2 (—)	14 (4)	2 (14)	36.0 ± 0.93	(50 ± 0.17)	10.418**
Six day	— (1)	— (14)	— (3)	—	(38 1.30)	—
Normal adult	— (8)	— (91)	— (1)	—	(29 0.83)	—

** Significant at 5% and 1% level.

Changes in PG cells:– In zero day fifth instar nymphs the PG cells appear to be transparent spherical, showing an outer thicker and an inner thinner layer of cytoplasm (Fig. 5). Very few granules are noticed during this period. During growth the transparency of the cell is lost and they become translucent. Shape of the cell changes from spherical to elliptical (Fig. 6); cytoplasmic vacuoles and granules begin to appear from second day onwards. PG cell volume as well as nuclear size gradually increases. Maximum size is noticed on the fifth day (Table 6). No significant difference has been noticed in the volume of PG cells between males and females. PG cell nuclear size increases on 2nd day and then decreases and again a still higher increase is noticed on sixth day (Table 6).

The maximum volume of CA and PG cells is not coincident but is successive. Volume of CA is maximum on the fifth day whereas that of the PG cells is attained on second and sixth days. Maximum neurosecretory index observed on the fifth day is coincident with CA enlargement.

Effect of exogenous juvenile hormone analogue (JHA) on the neuroendocrine system of the fifth instar nymphs:—

General effect on the insects: Topical application of juvenile hormone analogue (0.25 μg of ZR–777) just after emergence of the fifth instar nymph, produces sixth instar nymph instead of the normal adult. Some of the important external features affected by JHA are the abdominal spots, tarsal number and size of the wing pads. In the sixth instar nymphs the abdominal spots

TABLE 8. Changes in cell and nuclear size of A–cell of fifth instar nymphs during development. ZR–777 (0.25 μg) was applied on zero day. Control values are given in brackets (Mean of 10 observations).

Age of fifth instar nymphs (days after emergence)	A–cell size in μm		t value	A–cell nuclear size in μm		t value
Zero day (four h)	17.1 ± 0.49	(17.3 ± 0.35)	0.332	4.4 ± 0.35	(5.8 ± 1.07)	2.325*
One day	18.1 ± 0.51	(18.9 ± 0.49)	1.131	4.4 ± 0.33	(6.1 ± 0.68)	3.642**
Two day	19.4 ± 0.55	(19.8 ± 0.43)	0.573	4.8 ± 0.34	(7.3 ± 0.48)	4.250**
Three day	22.3 ± 0.47	(22.5 ± 0.56)	0.273	5.2 ± 0.29	(9.4 ± 0.29)	10.241**
Four day	21.6 ± 0.50	(24.5 ± 0.54)	3.940**	4.3 ± 0.29	(11.2 ± 0.26)	17.715**
Five day sixth instar nymph	19.4 0.45	(26.5 0.44)	10.486**	3.9 0.26	(7.1 ± 0.29)	8.216**
Six day	—	(21.9 ± 0.54)	—	—	(6.5 ± 0.43)	—
Normal adult	—	(21.2 ± 0.46)	—	—	(5.3 ± 0.43)	—

* Significant at 5% level.

** Significant at both 5% and 1% level.

and the wing pads persist and the tarsi are two in number.

Changes in A-Cell:— As early as four hours after ZR-777 treatment, the neurosecretory index of A-cells increases to 34 while it is only 23 in control insects. Quantitative changes in the NSM in the A-cells are represented in Table 7.

In treated insects the neurosecretory material increases upto second day, decreases on third day and again increases on fourth day. But on moulting, the value of neurosecretory index decreases slightly. In control insects maximum neurosacretory material is observed on fifth day and then it steadily decreases till moulting (Table 7).

A-cell size and its nucleus also show changes more or less parallel to that of the neurosecretory index. In treated insects maximum cell size is noticed on third day and in control insects it is on fifth day. Sixth instar nymphs show a smaller neurosecretory cell and nucleus compared to the controls (Table 8, Fig. 14).

In ZR-777 treated insects from four hour onwards the tritiated glycine incorporation increases. Number of grains in the perikaryon of the cell increases gradually. Maximum (clumped) grains in the perikaryon of the cells are noticed on fourth and sixth hour of second day (Figs. 7, 8). After second day the number of grains decreases in cell perikaryon and on third day the number of grains on hot control cells appears to be more than in the experimental insect. In the ZR-777 treated nymphs grain count again increases on fourth day, seen as clumps and grain density decreases on moulting ie., on the fourth and sixth hour of fifth day. On every day between eighth and sixteenth hour, the

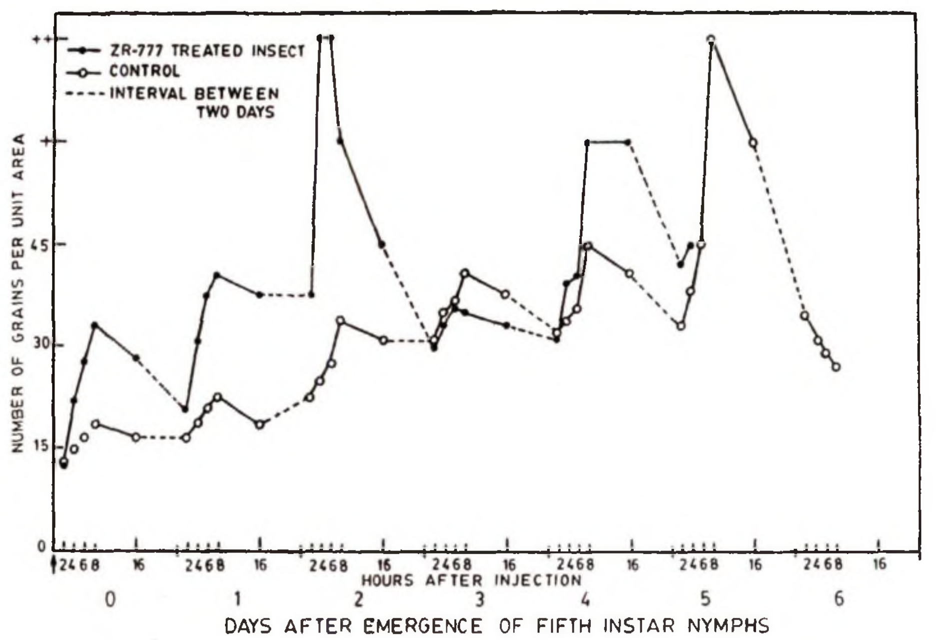

Fig. 8. Uptake of H^3 glycine by the A-cells of insects (1) treated with ZR-777 (0.25 μg) and by (2) control insects each point represents mean of 10 values. Arrow denotes time of application of ZR-777 or acetone as the case may be.

number of grains decreases regularly upto sixth day. Thereafter a general decrease is noticed (Fig. 8).

After tritiated thymidie injection very few grains are noticed in A-cells in the ZR-777 treated and in hot control insects. No significant difference in the grain counts between the A-cells and other neurons of the brain is noticed (Fig. 9).

Changes in CA:- ZR-777 reduces the volume of CA by 26% within four hours after the treatment. However during the growth period the volume of CA of treated insects increases upto fourth day and on fifth day the treated insects moult into sixth instar nymphs. At this time a 13% reduction in CA volume is noticed. In control insects the volume of CA increases to fifth day and slowly reduces till moult (Table 9).

Four hours after ZR-777 treatment CA shows very small nuclei (1.5 μm) and in control it is 1.8 μm. During development, always in treated insects in the CA nuclei are smaller in size (Table 9).

Incorporation of tritiaed glycine in CA of ZR-777 treated insects was found to be very slow. Maximum incorporation was noticed on the fourth day whereas in hot control insects from zero day onwards glycine incorporation increases and is maximum at sixth and eighth hour of fifth day (Fig. 10). From eighth hour of fifth day onwards again the rate of incorporation falls and is continued till moulting. Tritiated thymidine incorporation is negligible in CA (Fig. 11).

Changes in PG cells:- JHA treated insects exhibit large PG cells compared to the controls. During development volume of

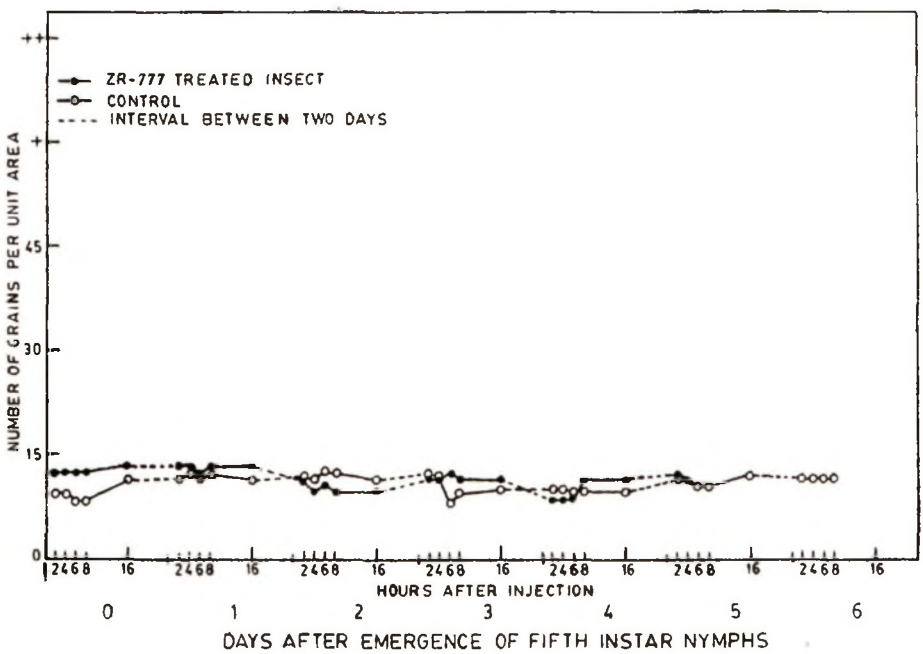

Fig. 9. Uptake of H^3 thymidine by the A-cells of insects (1) treated with ZR-777 (0.25 μg) and by (2) control insects. Each point represents mean of 10 values. Arrow denotes time of application of ZR-777 or acetone as the case may be.

TABLE 9. Changes in volume and nuclear size of corpus allatum of fifth instar nymphs during development. ZR-777 (0.25 μg) was applied on zero day. Control values are given in brackets (Mean of 10 observations).

Age of fifth instar nymphs (days after emergence)	Volume of CA in μm^3	t value	Nuclear size of CA cell in μm (Mean of diameter)	t value
Zero day (four hour)	5474 ± 103.3 (7412 ± 112.7)	12.677**	1.6 ± 0.17 (1.8 ± 0.17)	0.832
One day	7211 ± 102.3 (9786 ± 125.4)	15.913**	1.9 ± 0.14 (2.0 ± 0.17)	0.454
Two day	9185 ± 113.6 (10952 ± 118.6)	10.759**	2.3 ± 6.21 (2.9 ± 0.14)	2.377*
Three day	9753 ± 112.2 (17880 ± 107.3)	52.348**	2.9 ± 0.17 (4.1 ± 0.17)	4.991**
Four day	10646 ± 108.7 (25164 ± 129.4)	85.907**	3.7 ± 0.17 (5.0 ± 0.22)	2.606**
Five day sixth instar nympth	9163 ± 122.6 (34868 ± 113.7)	153.731**	2.7 ± 0.27 (5.6 ± 0.24)	8.028**
Six day	— (19936 ± 103.9)	—	— (4.7 ± 0.17)	—
Normal adult	— (15378 ± 115.6)	—	— (4.1 ± 0.23)	—

* Significant at 5% level.
** Significant at both 5% and 1% level.

TABLE 10. Change in cell volume and nuclear size of prothoracic gland cells of fifth instar nymphs during development. ZR 777 (0.25 μg) was applied on zero day. Control values are given in brackets (Mean of 10 observations).

Age of fifth instar nymphs (days after emergence)	Volume of PG cell in μm^3	t value	Nuclear size of CA cell in μm (Mean of diameter)	t value
Zero day (four hour)	48532 ± 1128 (30016 ± 1031)	12.116**	15.1 ± 0.23 (14.1 ± 0.16)	3.569**
One day	66107 ± 1242 (61971 ± 1012)	2.582**	16.7 ± 0.30 (15.4 ± 0.20)	3.605**
Two day	126405 ± 1715 (81675 ± 1825)	22.388**	22.4 ± 0.35 (24.1 ± 0.18)	4.319**
Three day	89129 ± 1624 (67942 ± 898)	11.417**	21.7 ± 0.36 (16.8 ± 0.22)	11.614**
Four day	79945 ± 1315 (53248 ± 1511)	13.328**	20.3 ± 0.18 (16.1 ± 0.16)	17.439**
Five day	75976 ± 1121 (54921 ± 1121)	13.281**	19.1 ± 0.28 (16.3 ± 0.25)	7.459**
Sixth instar nympth				
Six day	— (159281 ± 891	—	— (26.4 ± 0.31)	—
Normal adult	— (91212 ± 1311)	—	— (19.8 ± 0.30)	—

* Significant at 5% level.
** Significant at both 5% and 1% level.

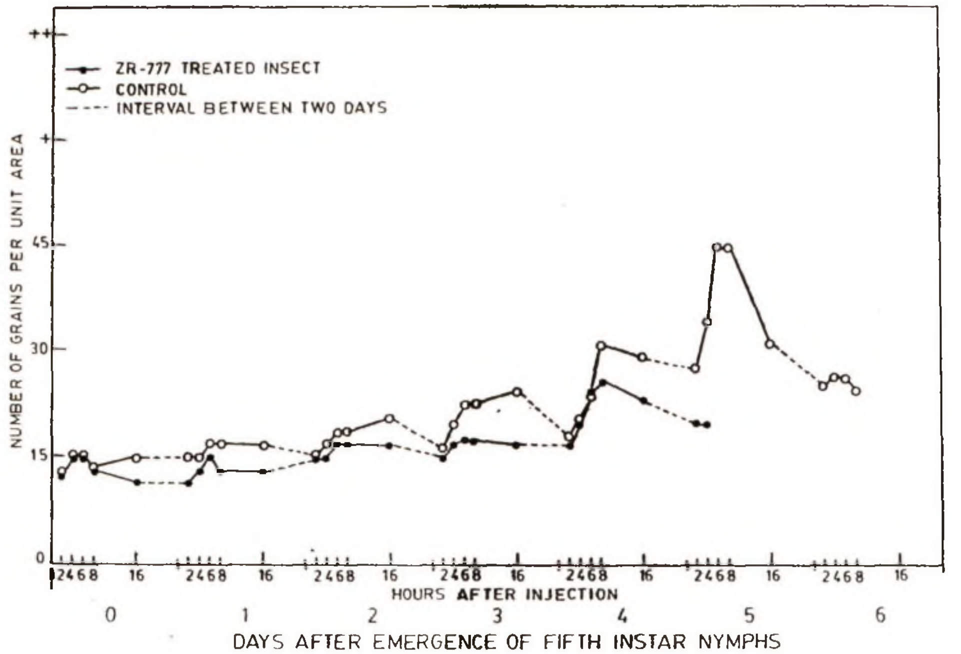

Fig. 10. Uptake of H^3 glycine by corpus allatum of insects (1) treated with ZR–777 (0.25 μg) and (2) by control insects. Each point represents mean of 10 values. Arrow denotes time of application of ZR–777 or acetone as the case may be.

PG cells in treated insects increases steeply and maximum volume is observed on the second day (126405 μm^3) whereas in control maximum PG cell volume is noticed on sixth day (Table 10). The PG cell nucleus also follows the same pattern (Table 10). Even after moulting into sixth instar nymph PG cells of ZR–777 treated insects remains sphereical whereas that of the control insects changes to elliptical shape progressively and show more cytoplasmic granules.

Tritiated glycine incorporation in PG cells is very significant and continuous. Maximum grains are noticed on sixteenth hour of second day (Fig. 12) in ZR–777 treated insects whereas in hot control maximum incorporation is noticed on sixth and eighth hour of fifth day.

Thymidine incorporation in treated and control insects is negligible (Fig. 13). Topical application of ZR–777 has an inhibitory effect on neurosecretory A–Cell; it reduces both A-cell as well as its nuclear size. ZR–777 also does reduce the activity of CA. Even then CA remains active; the inhibitory effect on CA is very clear from its nuclear size and volume. PG gland is the only endocrine gland which is stimulated by ZR–777. Positive action of ZR–777 is clear by the cell shape and size as well as the single longer activity peak found in PG cells (Fig. 14).

DISCUSSION

Based on the differences in stainability, cell size and nuclear size, three types of neurosecretory cells have been observed in the fifth instar nymphs of *Dysdercus cingulatus*. A–cells are most conspicuous; B-cells are smaller in size than A–cells. C–cells

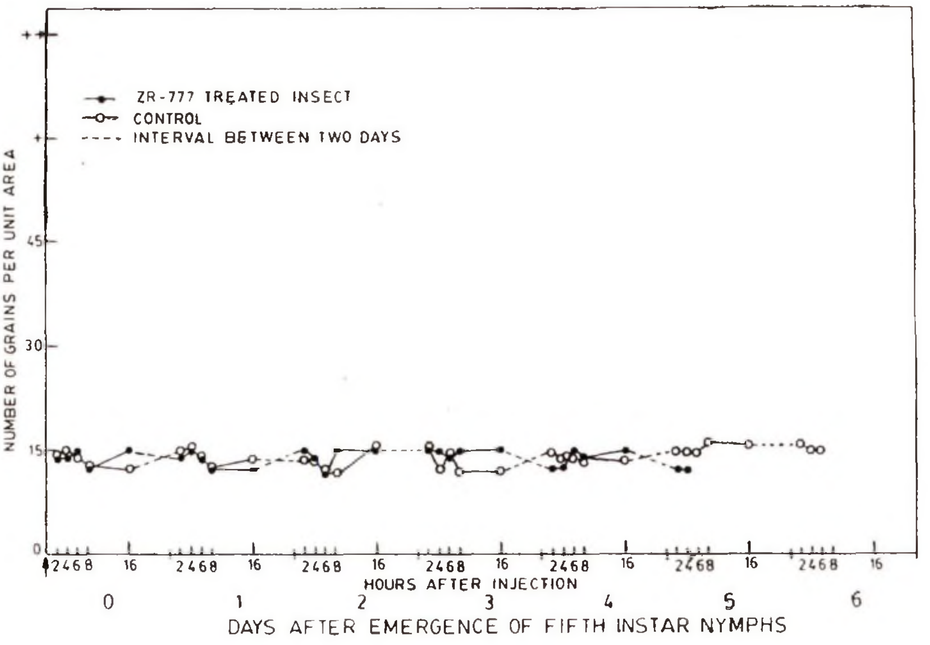

Fig. 11. Uptake of H^3 thymidine by corpus allatum of insects (1) treated with ZR-777 (0.25 μg) (2) and by control insects. Each point represents mean of 10 values. Arrow denotes time of application of ZR-777 or acetone as the case may be.

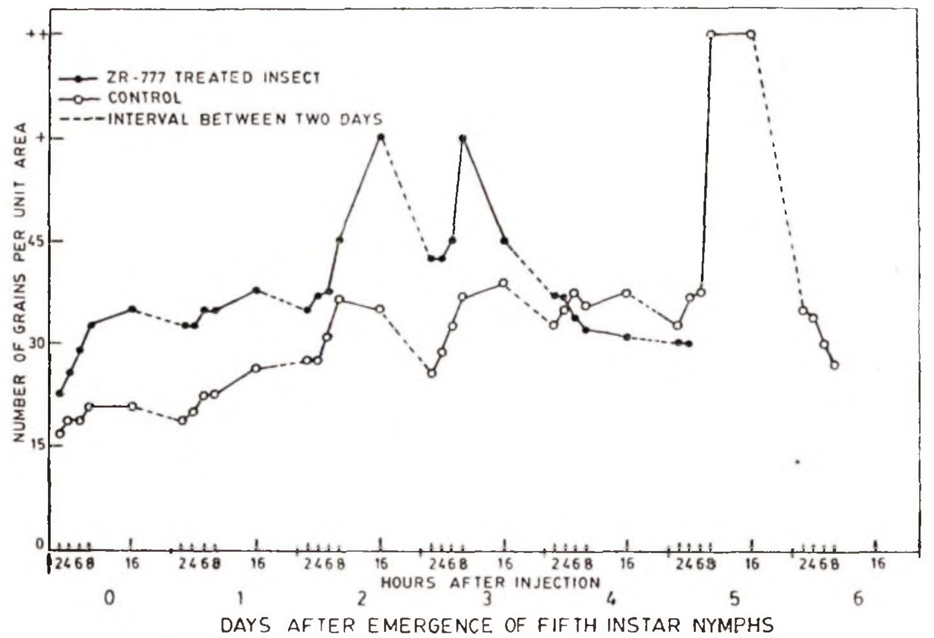

Fig. 12. Uptake of H^3 Glycine by prothoracic gland cells of insects (1) treated with ZR-777 (0.25μg) and (2) by control insects. Each point represents mean of 10 values. Arrow denotes time of application of ZR-777 or acetone as the case may be.

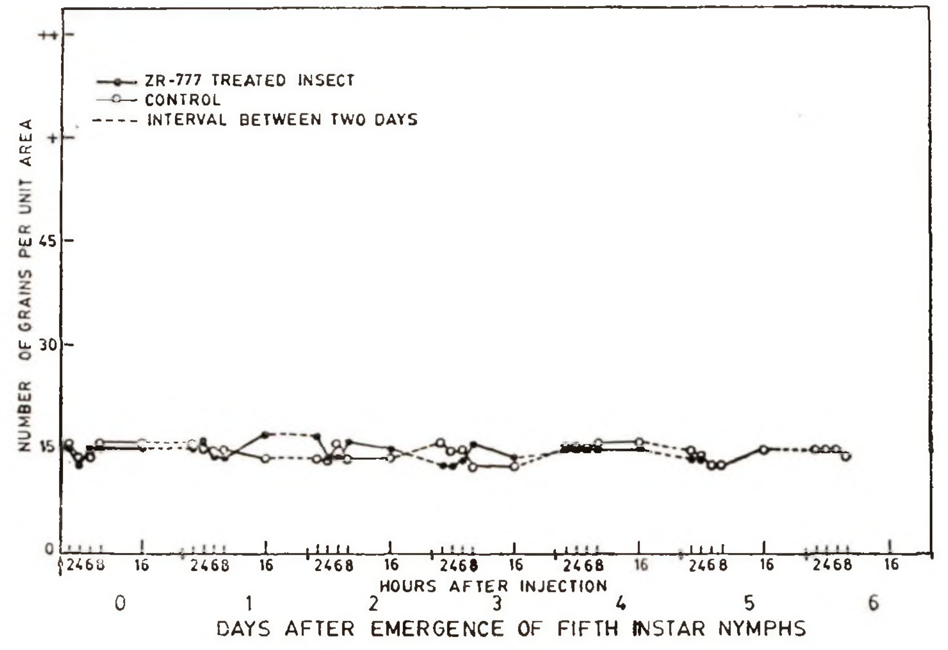

Fig. 13. Uptake of H^3 thymidine by prothoracic gland cells of insects (1) treated with ZR-777 (0.25 μg) and (2) by control insects. Each point represents mean of 10 values. Arrow denotes time of application of ZR-777 or acetone as the case may be.

are seen between the two groups of A-cells and are elongated in shape. The neurosecretory cells as well as corpus cardiacum and copus allatum are comparable to those in the adult of these species except in their size difference (JALAJA & PRABHU, 1977). As in the adult, CC does not function as a neurohaemal organ in nymphs also. The axons from neurosecretory A-cells can be traced to the aorta bypassing CC.

According to WELLS (1954) the prothoracic glands of *D. cingulatus* are compact. But the present study revealed their nature to be of loosely arranged cells and the characters have been established by transplantation studies (JOSEPH & PRABHU, 1977). Axon endings of the A-cells in the wall of the aorta (NHO) contains comparatively little secretory material at any time, when compared to the total quantity of material in the A-cell perikarya of the pars intercerebralis unlike that in *Leptinotarsa decemlineata* (SCHOONEVELD, 1970). So a quantitative analysis of secretory material contained in the axonal endings in the aortal wall in *D. cingulatus* was not attempted during the present study. It is recognised that cytological, histological and histochemical studies give only a static picture to predict whether the gland is active or inactive. Even though there are differences of opinion regarding the state of the activity of the NSC, CA and PG an increase in the size of the cells and nuclei and that of the gland can be taken as a sign of synthetic activity of the neurosecretory A-cell, CA and and PG (GABE & ARVY, 1961; RENSING, 1966; SCHOONEVELD, 1979; UNNITHAN 1972; TOBE & STAY, 1985) especially when activity cycle of the gland is followed closely, as in the present studies.

During the fifth instar life of the insect the size of the nuclei of the A-cells, which was very small, increases and attains maximum size on 3rd day and thereafter the size decreases. The neurosecretory cell size however reaches the maximum a day after (i.e., on day four) and both subsequently decrease gradually. The neurosecretory index attains maximum value one more day afterwards (i.e., in day five) and decreases thereafter. From this data it follows that the nuclear synthetic activity attains maximum on day three leading to maximum synthetic activity of the cell cytoplasm on day four and maximum accumulation of neurosecretory material in the cell on day five. The material accumulated in the A-cell till day five is gradually released before the animal embarks on to the next moult. Only one activity cycle for A-cells could be observed during the fifth instar nymphal period. The release of neurosecretory material could have started at any time after initiaton of nuclear activity which started even from the beginning of the fifth instar nymphal life, but there was clearly a spurt of nuclear activity on day three which apparently resulted in more synthetic activity of the neurosecretory material than release can cope and hence the accumulation of the synthesized material in the perikarya resulting in the peak accumulation of the synthesized material on day five. The nuclear activity gradually decreases from its peak activity on day three and from that period there is apparently only release of material unaccompanied by synthesis and hence a sudden depletion after day five. It may also be noted that during the fifth instar there is not much of difference in

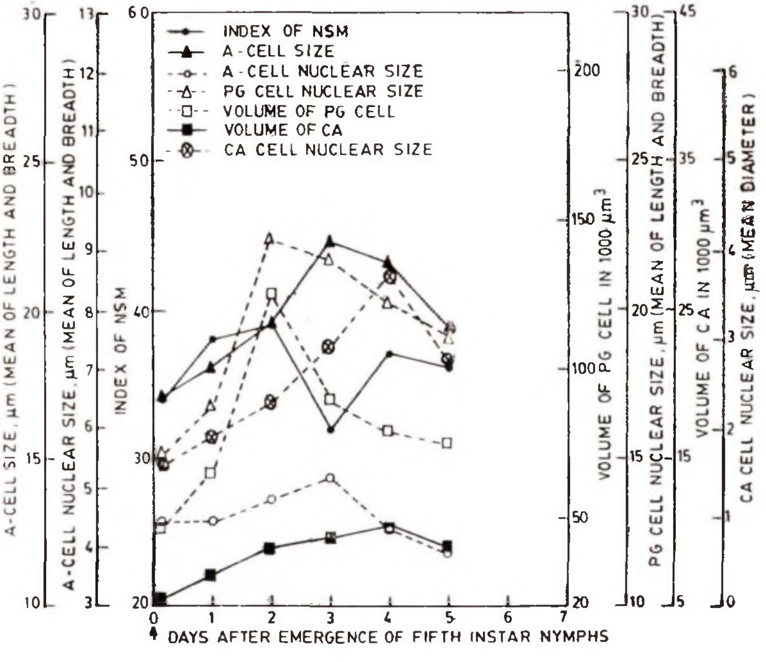

Fig. 14. Graph showing relationship between the index of neurosecretory material, A-cell size and its nuclear size, volume of cropus allatum and size of its nuclei, volume of prothoracic gland cell and size of its nuclei during development of fifth instar nymphs treated with ZR-777 (0.25 μg) on zro day. Each point represents mean of 10 values. Arrow denotes time of application of ZR-777 or acetone as the case may be.

activity between male and female, apparently because egg maturation and vitellogenesis are not involved.

During fifth instar nymphal period, the volume of the CA and the size of its cell nuclei are initially very low, they increase gradually attaining maximum size on day five. From then onwards the CA volume decreases steadily till on day seven. The area of CA and size of the nuclei also show an identical trend as that of neurosecretory cell during the fifth instar nymphal life.

Though increase in size of CA in itself cannot be taken to represent its higher activity, the increase in size of the gland exhibited by the nymphs of *D. cingulatus* during present study along with an increase in size of the nuclei can on firmer basis be interpreted to represent higher activity of the allatum. Thus one is on surer ground in presuming the activity of the allatum.

The PG cells register two peaks in their size during fifth instar nymphal life. The nuclear size of PG cells shows a corresponding change during this period. Morphometric and cytological criteria adopted by many workers to report the activity of the prothoracic gland cells (MALA *et al.*, 1974; SHAAYA & LEVENBOOK, 1982), would prompt one to consider that two peaks seen in the PG cell volume during fifth instar indeed represent two activity peaks (though again not necessarily release peaks). The earlier smaller peak was explained as the commitment peak by WATSON *et al.* (1987).

All the endocrine glands discussed above (the A-cells, the corpus allatum and prothoracic glands) start with comparatively low activity, at the beginning of the fifth instar and all of them subsequently increase in activity and hence it is very difficult to say which gland first affects which. As the prothoracic glands are made of loose cells exposed on all sides to haemolymph directly, one could presume that they would respond immediately to any change in the haemolymph mileau rather than any other endorine glands (neurosecretory cells and corpus allatum, most of the cells do not have direct easy access to the haemolymph). So it would appear that initial activity of the NSCs would first stimulate the PG cells and as the trophic hormone from the NSC would penetrate CA, the CA would also be stimulated, though slower than the PG cells. Prothoracotropic hormone of NSC is known to stimulate the PG (MALA *et al.*, 1977; SEHNAL & REMBOLD, 1985). There is sufficient evidence that pars intercerebralis neurosecretion stimulates CA activity in the adult *Dysdercus cingulatus* and in other insects (GRANGER & SEHNAL, 1974; HODKOVA, 1977; EDWARDS *et al.*, 1987). This machanism may be active in *D. cingulatus* nymph also.

Juvenile hormone of juvenoids are found to mimic the physiological effects of endogenous CA hormone (SLAMA *et al.*, 1974; NOVAK, 1975). Effects of treatment of JHA like ZR-512, ZR-515, ZR-619, ZR-777 etc. in various insects have been reported by GRANGER & SEHNAL (1974); NEGISHI *et al.* (1976), and SEHNAL & REMBOLD (1985). JH and JHA are maximally active at certain critical periods. In fifth instar nymphs of *Dysdercus cingulatus* the critical period, when tissues are sensitive to JHA was found to be between second and thrid day of 5th instar nymphal life. JHA applied, soon after emergence, on fifth instar nymph is found to be most effective.

Topical application of juvenile hormone analogue ZR-777 results in the retention of neurosecretory material in the neuroseretory cells. Neurosecretory cell size and nuclear size are conspicuously smaller when compared to controls. CA is found to be

smaller in size than control insects. But during the developmntal period, gland shows increase in volume which suggests that the gland is active. The small size of corpus allatum can be attributed to low synthetic activity. The small nuclear size of corpus allatum also supports the low activity of the gland (TOBE & STAY, 1985). A slight release of hormone from CA can be inferred from the decreases of the volume from 5th day. Inhibition of corpus allatum activity by JHA has been reported earlier by RIDDIFORD & TURNER (1978) and SLAMA *et al.* (1974). It is explained that in presence of excess juvenile hormone, corpus allatum in the insect body shows compensatory behavour. The gland remains active (depending on the pesristance of the juvenile hormone used) but secretes less hormone as there is excess JH present already in the haemolymph (SLAMA *et al.*, 1974; BAKER *et al.*, 1986; EDWARDS *et al.*, 1987).

In the case of ZR–777 treated insects, the excess juvenile hormone titre in the blood itself acts as a feed back mechanism. It checks the activity of corpus allatum, i.e., an inhibitory effect is produced as reported by NIJHOUT & WILLIAMS (1974), whereas positive feed back between JH and CA have been reported by SIEW & GILBERT (1971). Present study shows that excess JHA in haemolymph inhibits the release NSM from NSC. Also the high titre of JHA in the blood directly acts on CA and reduces its activity.

During fifth instar nymphal period PG cells in *Dysdercus cingulatus* show two peaks of activity. When JHA was applied, within four hours the volume of PG cells doubles. On second day maximum volume of the cells is noticed. There is only one peak in the activity cycle after JHA treatment.

Kinoprene applied on *D. cingulatus* shows a prothoracotropic activity. A high titre of JHA in the initial period of fifth instar nymphs appears to stimulate release of hormone from PG cells. A lesser quantity of ecdysone is liberated from PG cells at an earlier time i.e., on the second day-presence of a high titre of JHA in the haemolymph and a very low quantity of ecdysone liberated from PG cells induce the insects to moult into suprenumerary insects.

When JHAs are applied to the animal, the earlier peak in the size of the PG not only heightens, but the higher activity is maintained for a longer period. However second, more prominent peak noticed in the normal fifth instar nymphal period or in control insects is missing in the experimental animal i.e., two peaks of PG activity merge into a single peak which is sustained for a longer part of the nymphal period. Nuclear activity also follows the same pattern.

It is reported that JH present in haemolymph prevents the breakdown of PG cells which usually takes places at the time of the adult moult (GILBERT & SCHNEIDERMAN, 1959; SIEW & GILBERT, 1971). In *Dysdercus cingulatus* also it is seen that in JHA treated insects even after moulting the PG cells do not lose their activity as indicated by their morphological appearance and size of the nucleus.

Changes in the neurosecretory A–cells are apparantly reflected in the radioautographic studies using tritiated glycine. Upto two days after ZR–777 treatment labelled glycine uptake is higher when compared to control apparently because the RNA already synthesized and present in the cells synthesize some neurosecreory material which is stimulated by ZR–777. However nuclear activity has already inhibited by ZR–777 and this becomes evident in two days after ZR–777 treatment. In control insects maximum tritiated glycine incorporation is noticed

on day five and is a gradual increase from zero day. This supports the morphometric evidences. Tritiated glycine incorporation in CA and PG cell (in control) also shows a correlation with morphometric changes observed in these glands. Very little difference in labellled thymidine incorporation between control and experimental insects, as well as from other neurons indicate that little DNA synthesis is taking place in the endocrine glands.

Radio-autographic studies support the views that ZR-777 inhibits A-NSC and CA and enhance activity of PG cells in *D. ciugulatus*.

In the light of present findings and keeping in view the mechanism known to exist among endocrine glands of various insects based upon the findings of different workers, a tentative mechanism of endocrine interrelationship in *Dysedercus cingulatus* in the last (fifth) nymphal instar is put forward.

A-cells from the brain produce two tropic hormones; (1) The prothoracotropic hormone which is rapid in its action as the prothoracic gland cells have direct access to haemolymph; (2) The allatotrophic hormone which activates the corpus allatum much slowly as this is a compact gland. The neurosecretory A-cells are activated apparently first in the cycle, and then the prothoracic glands get activated and its activity reaches a peak on the second day in response to the prothoracotrophic hormone from the A-cells. The ecdysone released acts on prothoracic gland cells themselves and inhibit prothoracic gland actvity.

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DIFFERENTIAL SUSCEPTIBILITY OF VARIOUS LIFE STAGES OF MOSQUITOES TO CERTAIN CHITIN SYNTHESIS INHIBITORS

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The overall efficacy of four chitin synthesis inhibitors, viz., diflubenzuron, penfluron, ethyl penfluron and Bay SIR 8514 was investigated in the larval, pupal and adult stages of mosquitoes, *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi*, and *An. culicifacies*. The present data evidently indicate that the test compounds were very toxic to immature stages of all the four species of mosquitoes and caused moderate to high mortality in various life stages. The mortality occurred either at the larval or pupal moults or at adult emergence, indicating that these compounds inhibited chitin synthesis during the moulting stage. Among the four compounds tested, penfluron was found to be the most toxic chemical to all the species of mosquitoes, followed by diflubenzuron and Bay SIR 8514. Also, anophelines were more susceptible than culicines in most of the treatments.

(*Key words:* chitin synthesis inhibitors, diflubenzuron, penfluron, Bay SIR 8514, anopheline mosquitoes, culicine mosquitoes)

INTRODUCTION

The insecticidal potential of benzoylphenyl ureas has been studied since 1970. These compounds seem to be promising insecticides due to their high activity on insects, very low toxicity to vertebrates and their relative selectivity in favour of the non-target arthropods (MARX, 1977; HEJAZI & GRANETT, 1986). Besides larvicidal activity, chitin synthesis inhibitors cause delayed toxicity symptoms such as inhibition of pupal formation and adult sterility at very low concentration (HEJAZI & GRANETT, 1986). Since previous studies have been mo tly confined to evaluating the larval susceptibility of mosquitoes to chitin synthesis inhibitors, it was considered important to assess the differential susceptibility of various life stages of mosquitoes to these compounds in order to develop control strategies. Therefore present investigations were commenced to evaluate the effects of four benzamide chitin synthesis inhibitors, viz., diflubenzuron, penfluron, ethylpenfluron and Bay SIR 8514 to various life stages of the mosquitoes, *Culex quiuquefasciatus, Aedes aegypti, Anopheles stephensi,* and *An. culicifacies.*

MATERIALS AND METHODS

Different life stages of mosquitoes, viz., larvae, pupae and adults were drawn from laboratory colonies of *Cx. quinquefasciatus, Ae. aegypti, An. stephensi and An. culicifacies* established from blood-fed females collected from the field in Delhi and maintained at $27 \pm 2°$ C and 85 ± 5 % RH and provided with 14 hours of artificial daylight using an automatic electronic dimmer (BAKSHI, et al., 1982). Three of the compounds, diflubenzuron (1- (4-chlorophenyl) -3-(2-6-difluorobenzoyl) urea), penfluron (2-6-difluoro) N-(4-trifluoromethyl phenyl)-amino

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carbonyl benzamide and ethyl penfluron were 90% pure and were supplied by Dr. A. B. BORKOVEC, USDA, Beltsville, U.S.A. Bay SIR 8514 (2-chloro-N((((4-trifluoromethoxy) phenyl) amino) carbonyl) benzamide) was obtained from Bayer (India) Limited, India. Stock solutions (1%) of the test compounds were made in acetone.

Synchronously hatched second, third and fourth instar larvae of each species of mosquitoes were treated with the chemicals according to the procedure adopted by WHO (1981). The mortality data were subjected to computer analysis of the regression of probit mortality on log dosage on TBM - 360/44 computer at the Computer Centre of University of Delhi. LC_{50}, LC_{90} and slope of the linear regression line were computed.

Pupae of non age (0–18 hours old) were collected and exposed for 4 hours to graded concentrations of the test compounds prepared in 250 ml of dechlorinated water. Parallel control treatment were performed. After 4 hours of exposure, pupae were separated and rinsed. The pupal mortality was scored. Surviving pupae were kept in mosquito cages for adult emergence. Only active adults were counted as surviving and others were counted as dead. The data was subjected to probit analysis using the computer as described earlier. The delayed effects of the test compounds to adults were assessed. After mating, eggs laid per female, percent egg hatch, larval survival and adult emergence were recorded.

Adult mosquitoes of all the four species were treated with the given compounds either by ingestion or by contact. Thus 20 newly emerged males and females each were allowed to feed on 10 per cent sucrose solution containing 10 ppm of test solution for 24 hours. Treatment by contact was assessed by two methods, either by exposing the adult mosquitoes to glass vials coated with the test compounds or to filter papers impregnated with the chemicals. The concentration of the test chemical was 0.165 μg/sq cm. Newly emerged 20 males or females were exposed to these vials for 2 h each separately. The control vials were having a coat of acetone only. The mosquitoes treated by either ingestion or contact were transferred to clean cages and were allowed to mate. The effect of chemicals on the fecundity of females, egg hatchability and larval survival were studied for the first two gonotrophic cycles.

In all above treatments all the abnormalities noticed during different life stages were critically examined. These abnormalities were identified and characterised during development of the larvae and pupae and computed at LC_{50} and CL_{90} values.

RESULTS AND DISCUSSION

The larval instars of all the species of mosquitoes were highly susceptible to the chitin synthesis inhibitors as evident from the extremely low LC_{50} and LC_{90} values (Table 1) of the four compounds tested. Penfluron was found to be the most toxic compound, *An. culicifacies* being the most susceptible species followed by *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Besides penfluron, diflubenzuron was also found to be extremely toxic to larvae of all the species of mosquitoes, Bay SIR 8514 and ethyl penfluron being less toxic than either of the two compounds. It was also observed during the present study that second and third instar larvae were much more susceptible to test compounds than fourth instar stage. This suggests that younger larvae with a longer food intake period are likely to ingest and retain more of the test compounds. Similar effects have been reported by AMIN & WHITE (1984), BEEVI & DALE

(1980), GUJAR & MEHROTRA (1989) and MULLA et al. (1974).

Table 2 shows LC_{50} and slope values when pupae of different species of mosquitoes were exposed to the test compounds for 4 h. Pupae of *Ae. aegypti* and *Cx. quinquefasciatus* although equally susceptible, had higher LC_{50} values than the anopheline pupae. Penfluron was most toxic to pupae of all species, closely followed by diflubenzuron. The test compounds affected the adults emerged from the treated pupae. Fecundity of females was considerably reduced in such adults while no significant effect was noticed on the egg hatchability or larval survival of F_1 generation. The reduction in fecundity may be due to some mechanisms inhibiting oviposition in treated females (SOLTANI et al., 1984).

TABLE 1. Larval LC 50* (in ppb) of four species at mosquitoes (different instars) treated with four chitin synthesis inhibitors.

Test Compound	II instar		III instar		IV instar	
	LC_{50}	Slope	LC_{50}	Slope	LC_{50}	Slope
Cx. quinquefasciatus						
Diflubenzuron	3.25	0.363	1.00	0.452	34.66	0.031
Penfluron	0.69	1.410	0.73	1.263	12.95	0.083
Bay SIR 8514	4.21	0.290	5.27	0.215	17.47	0.078
Ethyl penfluron	20.93	0.074	15.35	0.073	73.44	0.018
Ae. aegypti						
Diflubenzuron	0.85	1.337	0.75	1.591	2.89	0.617
Penfluron	0.22	5.141	0.35	3.082	1.09	0.730
Bay SIR 8514	3.07	0.280	2.82	0.284	6.25	0.246
Ethyl penfluron	13.36	0.119	11.69	0.166	22.26	0.075
An. stephensi						
Diflubenzuron	0.82	0.817	0.80	1.040	1.22	0.879
Penfluron	0.69	1.318	0.69	1.180	1.07	0.979
Bay SIR 8514	0.81	1.464	1.13	1.065	2.12	0.216
Ethyl penfluron	23.44	0.033	28.84	0.017	33.26	0.027
An. culicifacies						
Diflubenzuron	0.24	6.175	0.23	8.359	2.14	0.697
Penfluron	0.35	4.836	0.07	2.312	1.09	0.833
Bay SIR 8514	6.86	0.133	7.59	0.133	19.72	0.056
Ethyl penfluron	7.60	0.175	11.44	0.084	24.70	0.046

* Corrected mortality calculated by Abbott's formula.

TABLE 2. Effect of three chitin synthesis inhibitors on pupae* and the subsequent generation (F_1) after treatment in four species of mosquitoes.

Test compound	LC_{50}* (ppm)	Slope	F_1 generation			
			Eggs laid/female	% Egg hatch	% Larval survival	% Adult emergence
Cx. quinaquefasciatus						
Control			70.83	82.26	83.19	89.79
Diflubenzuron	2.18	0.40	22.60	77.49	84.48	87.47
Penfluron	1.27	0.71	37.30	87.94	86.24	94.30
Bay SIR 8514	8.83	0.18	33.90	93.78	88.65	89.25
Ae. aegypti						
Control			150.60	87.60	89.21	76.14
Diflubenzuron	2.10	0.41	46.49	93.72	85.21	83.05
Penfluron	1.31	0.83	56.98	95.24	85.11	90.71
Bay SIR 8514	8.88	0.19	39.12	70.15	59.13	77.70
An Stephensi						
Control			67.47	89.38	85.91	87.41
Diflubenzuron	1.29	0.50	34.97	85.51	80.68	73.37
Penfluron	0.97	1.20	26.13	87.57	75.55	67.26
Bay SIR 8514	1.40	0.76	54.67	89.31	83.28	79.74
An. Culicifacies						
Control			44.36	86.08	85.25	85.36
Diflubenzuron	0.89	1.13	23.80	68.41	72.88	60.08
Penfluron	0.90	1.24	11.46	79.62	72.00	58.33
Bay SIR 8514	1.28	0.63	26.40	66.70	74.01	76.33

* Corrected mortality as calculated by Abbott's formula.

+ Age of the pupae: 0–18 hrs.

It was observed in treatment of adult mosquitoes with the test compounds either by ingestion or by contact that both type of treatments were almost equally effective and produced a significant reduction in the number of deposited eggs as well as inhibition in egg hatchability (Table 3). Reduction in fertility was more than the larval mortality in all the crosses. Besides, a greater inhibition of egg hatch resulted when treated male and female adults were mated to each other. Similar results on fecundity, inhibition of egg hatch and larval mortality were reported by MIURA & TAKAHASHI,

TABLE 3. Effect of three chitin synthesis inhibitors on fecundity and fertility in four species of mosquitoes treated as adults either by ingestion* or contact.+

Treatments	Diflubensuron		Penfluron		Bay SIR 8514	
	% Reduction in Fecundity	% Reduction in Fertility	% Reduction in Fecundity	% Reduction in Fertility	% Reduction in Fecundity	% Reduction in Fertility
Cx. quinquefasciatus						
Control	—	16.2	—	16.2	—	16.2
Treated diet	18.7	69.9	79.3	54.6	57.9	42.1
Treated filterpaper	70.3	25.0	19.8	12.7	55.7	39.7
Treated glass vial	55.9	79.1	—	61.9	50.8	75.5
Ae. aegypti						
Control	—	21.2	—	21.2	—	21.2
Treated diet	3.5	85.8	59.5	47.0	86.2	71.3
Treated filterpaper	49.9	85.6	71.9	47.4	77.1	83.1
Treated glass vial	15.4	86.0	79.7	70.7	73.4	79.5
An. stephensi						
Control	—	14.3	—	14.3	—	14.3
Treated diet	50.8	63.7	24.3	70.9	24.9	67.4
Treated filterpaper	19.5	38.8	—	66.2	45.2	68.1
Treated glass vial	32.1	67.5	4.0	73.2	60.1	60.2
An. culicifacies						
Control	—	10.8	—	10.8	—	10.8
Treated diet	40.3	69.6	46.5	68.9	52.4	67.4
Treated filterpaper	41.4	63.3	52.4	69.0	27.6	63.1
Treated glass vial	23.8	64.3	45.5	72.3	46.2	65.2

* Concentration : 10 μg/ml in 10% sucrose.

\+ Concentration : 0.165 μg/sq cm.

(1978), KNAPP & HERALD, (1983). KOEHER & PATTERSON (1989) and SARASUA & SANTIAGOALVAREZ (1983). When total effects were compared in all the species of mosquitoes, penfluron and diflubenzuron were almost equally effective, closely followed by Bay SIR 8514. Among different species, *Ae. aegypti* adults were most susceptible to test compounds, followed by *Cx. quinquefasciatus*, *An. stephensi* and *An. culicifacies* (Table 3).

Treatment of different larval instars with the various doses of chitin synthesis

TABLE 4. Larval and pupal morphological deformities in four species of mosquitoes treated at third instar larvae continuously till adult emergence with LC_{50} and LC_{90} concentrations of chitin synthesis inhibitors.

Species	Test compound	% Larval deformities at LC_{50}	% Larval deformities at LC_{90}	%Pupal deformities at LC_{50}	%Pupal deformities at LC_{90}
Cx. quinquefasciatus	Diflubenzuron	6	72	44	20
	Penfluron	20	90	30	2
	Bay SIR 8514	22	80	30	12
Ar. aegypti	Diflubenzuron	16	80	34	12
	Penfluron	24	86	28	8
	Bay SIR 8514	26	66	26	26
An. stephensi	Diflubensuron	16	66	32	22
	Penfluron	22	86	28	4
	Bay SIR 8514	12	76	40	14
An. culicifacies	Diflubensuron	24	74	28	16
	Penfluron	24	74	24	16
	Bay SIR 8514	18	76	32	12

inhibitors impaired the process of moulting. The degree of inhibition of moulting and the severity of morphological deformities increased with the increase in the concentration of compound used. At lower concentrations the treated larvae were able to complete pupal moulting and sometimes adult development, which gave higher proportion of pupal than larval deformities. However at higher doses, most of the treated larvae died before attaining the pupal stage, resulting in higher frequency of larval than pupal abnormalities (Tale 4). The incomplete moulting and morpholcgical deformities observed during the present study could have been due to blockage of deposition of chitin in the endocuticle as has been reported in *P. brassicae* (POST & VINCENT, 1973), in *N. serinopa* (SUNDARAMURTHY & SANTHANAKRISHNAN, 1979) and in *M. domestica* (SHAFI et al., 1987).

As the conventional insecticides are becoming less popular due to rapid development of resistance and their ability to pollute the environment, the present studies clearly indicate that chitin synthesis inhibitors can be effectively used in mosquito control programmes.

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PATTERNS IN THE POPULATION DRIFT AND SEED RESOURCE UTILIZATION OF *SPILOSTETHUS HOSPES* (FAB.)

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Spilostethus hospes **(Fab.) utilizes efficiently the seeds of** ***Solanum melongena, S. torvum, Lycoperscicon esculentum, Calotropis gigantea, Vernonia cinerea*** **and** ***Euphorbia hirta.*** **Crop plants being seasonal, their availability in the habitat throughout the year cannot be depended. The perennial weed** ***C. gigantea*** **serves as reservoir and the bugs thrive on them until other host plants appear in the habitat.** ***V. cinerea*** **springs up immediately after the rains and serves as an alternate host. The paper presents an in depth analysis of the patterns observed in the population drift based on a three year analysis of the population fluctuation. Observations relating to the natural food resources of** ***S. hospes*** **and the efficiency of utilizaton of these resources are also presented.**

(*Key words:* *Spilostethus hospes*, population drift, resource utilization, Lygaeidae)

INTRODUCTION

Lygaeids, predominently being seed feeders, are dependent on seed resources that are often highly variable in space and time. While many phytophagous insects can utilize only a narrow range of plant species, seed feeding insects face the additional problem of tracking spatial and temporal changes in the distribution of individual plants that bear seeds (JANZEN, 1971). *Spilostethus hospes* (Fab.) utilizes such crops as *Solanum melongena, Lycoperscicon esculentum* and weeds as *Calotropis gigantea, Vernonia cinerea* and *Euphorbia hirta* for feeding and breeding (ANANTHAKRISHNAN *et al.*, 1983). The crops being seasonal, their continued availability as a food resource cannot be depended. Therefore, the bugs, either should show great mobility so as to search for seed-bearing host plants or should be able to utilize alternate hosts in the vicinity. The seed feeding bug, *Lygaeus equestris* (L), changes and extends its food plant spectrum during its life cycle (KUGELBERG, 1974), while *Oncopeltus fasciatus* Dallas showed great dispersibility associating strongly with fruiting plants (BLAKLEY, 1980). As resources vary, populations of consumers often respond with a time lag, a phenomenon called 'tracking inertia' (WIENS, 1984). The dynamics of seed–seed predator system thus depend primarily on how seed predator population track their variable resources in time and space (DESTEVEN, 1983; SOLBRECK & SILLEN–TULLBERG, 1986). An attempt is made here to study the efficiency of utilization of the natural food resources by *S. hospes* and to analyse the causes for the drift in the population with emphasis on the possible role of weeds as reservoirs for *S. hospes*.

MATERIAL AND METHODS

Adults and nymphs of *S. hospes* were collected from various host plants and reared in the laboratory as per the methodology of ANANTHAKRISHNAN *et al.* (1983). Observations were made on the duration of postembryonic development, resource utilization and fecundity when fed on different seeds

according to the methodology of SANJAYAN & ANANTHAKRISHNAN (1987). Measurements of the different body parts was determined with the aid of a stereomicroscope with a measuring scale. Population dynamics was studied by weekly sampling of the population over a period of three years. Census was taken on the hosts *C. gigantea* and *V. cinerea* following the methodology adopted for *O. fasciatus* by SAUER & FIER (1973). To analyse the relation between the population fluctuations and the abiotic factors of the environment, meteorological data with respect to the maximum and minimum temperatures, relative humidity and rainfall were obtained from the nearest meteorological station. Analysis of the data were according to SOLBRECK & SILLEN – TULLBERG (1990) where bug densities were 10 log transformed before analysis. Zero samples of bugs have been omitted from analysis of bug densities. This was done to avoid problems of spurious correlations occurring as a result of log (n+1) trasformation. Instead, zero samples have been used in the separate analysis of colonization on host plants over the years. Simple and multiple regressions have been employed as done by SOLBRECK & SILLEN – TULLBERG (1990) for *L. equestris*.

OBSERVATIONS AND DISCUSSION

Natural food resource of S. hospes : *S. hospes* feeds on a wide variety of plants belonging to the families Asclepiadaceae Euphorbiaceae, Solanaceae, Compositae and Malvaceae. The more common plants serving as natural food resource for *S. hospes* are *Solanum torvum*, *S. melongena*, *L. esculentum*, *C. gigantea*, *V. cinerea*, *E. hirta* and *Physalis minima*. The bug, therefore, is polyphagous but feeds specifically on the generative parts of the plant such as the ovulae of the flowers as well as unripe and ripe seeds, although occasionally they may be seen sucking the leaves and stems, obviously to get water.

Efficiency of utilization of the natural food resource: Laboratory studies on the host preference indicate the Asclepiadaceous plant *C. gigantea* to be the most preferred host in terms of the biological response of *S. hospes*.

Table 1 shows that the rate of post-embryonic development is significantly affected when fed on different host seeds. *S. melongena* appears to be the most nutritive host as far as the rate of development and fecundity are considered, followed by *C. gigantea*. There was no significant difference in the total time taken to develop from the egg to the adult when the bugs were fed on *V. cinerea* or *E. hirta*. MUKHOPADHAY (1985) observed the rate of development and fecundity of *S. pandurus* to enhance considerably when the bugs were kept on a diet of *Calotropis* seeds and its sap while a diet with a combination of sunflower seed and water seldom instigated egg laying although it maintained steady nymphal development. SANJAYAN & ANANTHAKRISHNAN (1987) have attributed the difference in the performance of *S. hospes* fed on different seeds to the difference in the nutritive value of the seeds. Seeds of higher nutritive value resulted in faster rates of post-embryonic development, better growth rates and higher fecundity. Studies on the efficiency of food utilization in terms of the quantitative intake of different seeds by *S. hospes* indicated an ECI (Efficiency of conversion of ingested food) value of 60.15% for *C. gigantea* 55.79% for *V. cinerea* and 37.76% for *E. hirta*. The ECD (Efficiency of conversion of digested food) values ranged from 60–67%. This indicates that *S. hospes* efficiently digests the seeds of *C. gigantea*, *V. cinerea* and *E. hirta*; the highest value being for *C. gigantea*. This could account for the faster rate of post-embryonic

TABLE 1. Effect of host seeds on the duration of development of various nymphal stages.**

Host Plant	Incubation period *	First instar *	Second instar *	Third instar *	Fourth instar *	Fifth instar *	Total period *	Mean fecundity
Solanum melongena	6.5 ± 0.50 (6–7)	2.3 ± 0.77 (2–3)	5.6 ± 0.77 (4–7)	6.3 ± 0.47 (6–7)	7.7 ± 0.82 (7–9)	6.7 ± 0.47 (6–7)	35.1 ± 3.48 (31–40)	139.30 (52–157)
Vernonia cinerea	10.6 ± 0.48 (10–11)	6.6 ± .80 (6–7)	8.2 ± 1.23 (6–10)	7.4 ± 0.73 (6–8)	7.0 ± 0.73 (6–8)	9.0 ± 0.00 (9)	48.8 ± 4.01 (43–53)	46.83 (35–73)
Euphorbia hirta	8.6 ± 0.00 (8)	7.7 ± 1.01 (7–8)	5.8 ± 0.83 (5–6)	11.3 ± 2.30 (10–13)	6.7 ± 0.95 (6–8)	9.4 ± 0.69 (9–10)	48.9 ± 5.78 (45–53)	40.50 (38–43)
Calotropis gigantea	6.3 ± 1.00 (5–8)	6.5 ± 0.50 (6–7)	3.3 ± 0.47 (3–4)	7.0 ± 0.81 (6–8)	7.6 ± 0.80 (7–9)	6.9 ± 1.60 (6–11)	37.6 ± 5.18 (33–47)	98.80 (78–118)

* Days.
** Mean ± SD of 6 replicates.
Values in parentheses represent range.

ANOVA

Source	F calculated	F (table)	CD (.05 level)
Stage	1.954	2.9	—
Host plant	3.692	3.29	2.089

development, growth and fecundity being observed for individuals fed on *S. melongena* and *C. gigantea*, in addition to the better chemical nutrients being available from these seeds. RAMAN & SANJAYAN (1983) in studying the food utilization and reproductive programming of *Oxycarenus hyalinipennis* Costa showed that dietary schedules involving food limitation either in the form of starvation or diets of lower nutritive value profoundly affect the total egg production and the sequence of egg laying. *L. equestris* derive all the resources for egg production from feeding during the adult stage and during the larval feeding, proportionately more resource are devoted to developing the exoskeleton than to internal organs (SOLBRECK *et al.*, 1989). In the present study, seeds of different host plants elicited the feeding response of the bug differently and this resulted in different amounts of food intake and corresponding differences in the growth, development and fecundity.

Morphometric growth in relation to resource utilization: Growth refers to the irreversible quantitative changes in size and the nymphal period is pre-eminently one of growth. In insects there is a geometrical increase in size of the head width as well as linear measurements of cuticular structures during the progressive stages of development. Head width was measured for each instar stage of *S. hospes* fed on different host seeds and the progression factor calculated (Table 2). An overall mean growth rate from first instar to adult of 1.2725 when fed on *C. gigantea*, 1.2835 on *V. cinerea* and 1.251 on *E. hirta* was observed. These growth rates are close to that generalized for insects being 1.26. The head width measurements did not show significant differences on different host plants indicating that this could be used as a reliable parameter for fixing the developmental stage of the lygaeid. However, food resources had a profound effect on the total body length, the maximum growth being recorded on the most nutritive food (ANANTHAKRISHNAN *et al.*, 1983). An understanding of the growth rates has widespread application and reflects the subordination of organ size to physiological and ecological consideration. *L. equestris* females have larger body size than the males and fall easy prey to predator. Therefore selection pressure in this species has tended towards individuals with smaller body size (SOLBRECK *et al.*, 1989). Huxley's allometric formula $Y = bx^K$ was used to describe the growth of body parts of *S. hospes* (Table 3). The head width was taken as the standard for comparison as it was observed to be the most stable part of the body and least subjected to change in size owing to changes

TABLE 2. Head with measurements of *Spilostethus hospes* fed on different seeds.

Host	Instars					Adult		Progression Factor	
	I	II	III	IV	V	Male	Female	Male	Female
C. gigantea	0.536	0.782	1.020	1.224	1.836	1.938	2.040	1.109	1.119
V. cinerea	0.493	0.867	1.020	1.408	1.867	1.913	1.938	1.140	1.143
E. hirta	0.561	0.799	1.105	1.428	1.632	1.785	1.913	1.132	1.145

All values are in millimetres and represent mean of 10 replicates.

TABLE 3. Growth patterns of *S. hospes.*

Host	Parameters	Growth ratio (k)		Initial growth index (b)		Correlation coefficient (r)	
		Male	Female	Male	Female	Male	Female
C. gigantea	Total body length	6.255	6.086	—2.167	—2.037	0.986	0.991
	Rostrum length	1.983	2.282	+0.296	+0.039	0.960	0.975
	Total antennal length	2.785	2.923	—0.540	—0.676	0.983	0.987
V. cinerea	Total body length	5.712	5.699	—2.048	—2.043	0.975	0.976
	Rostrum length	2.130	2.415	—0.157	—0.404	0.974	0.940
	Total antennal length	2.618	2.757	—0.445	—0.569	0.954	0.941
E. hirta	Total body length	5.425	5.368	—1.499	—1.531	0.964	0.974
	Rostrum length	3.138	3.115	—1.105	—1.045	0.926	0.933
	Total antennal length	3.093	3.248	—0.982	—1.138	0.979	0.976

in physiological state of the individual. *S. hospes* shows positive allometry of the total body length, rostrum length and antennal length in relation to head width under all seed foods. An inverse relation between the growth ratio 'k' and the initial growth index 'b' was observed as has been shown for other insects by MATSUDA (1961). Host plants influenced the size of the body parts of *S. hospes* significantly although growth was allometric.

Population dynamics: *C. gigantea* and *V. cinerea* are two important weeds growing abundantly along the bunds of the irrigation fields and harbouring heavy populations of *S. hospes.*While *C. gigantea* is a perennial weed, *V. cinerea* springs up immediately after the monsoon and seeds become available from October to April each year. Pod setting in *C. gigantea* begins by late August and by November there is a high density of mature seeds. The analysis of the population fluctuation of *S. hospes* on these two hosts indicate interesting results. In the present study, on *C. gigantea,* the population showed a single peak every year during March/April (Fig 1) in contrast to the earlier observation of THANGAVELU (1978) and in support of MUKHOPADHYAY (1982). A sharp increase in population was followed by a sharp decrease during all the three years. The population curve depicted a different trend on *V. cinerea,* extending from October to May for the first two years and from December to August for the third year (Fig. 2) Unlike as in *C. gigantea,* where only a single peak was evident every year, several were evident on *V. cinerea* with special differences existing in the magnitude of the population as well. Peak population of *S. hospes* was observed during March/April although the density of available *C. gigantea* seeds was the highest in August. This indicates that population fluctuation is not solely governed by the seed density, but other environmental factors also have an important role. BLAKLEY (1980) observed populations of *Oncopeltus cingulifer* often on plants which lacked seeds. Their lower flight capacity and shorter intervals between

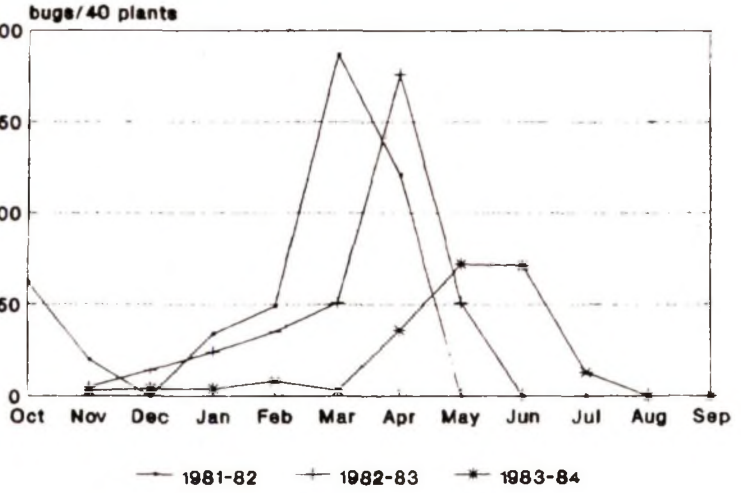

Fig. 1. Population fluctuation of *S. hospes* on *C. gigantea.*

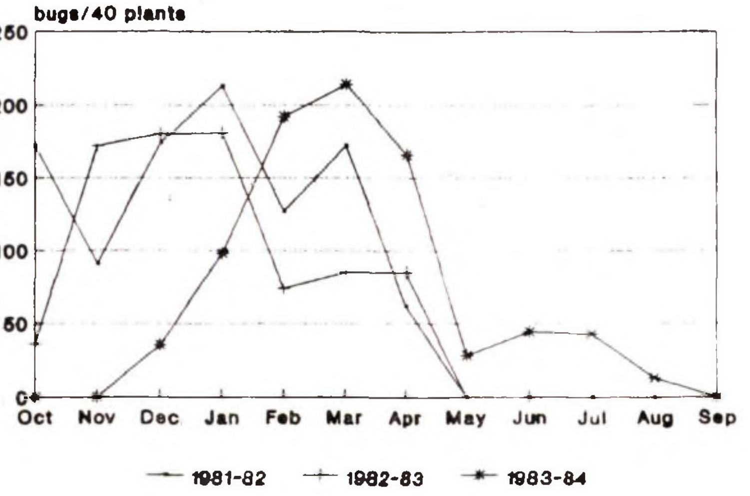

Fig. 2. Population fluctuation of *S. hospes* on *V. cinerea.*

successive clutches limit the mobility of egg-laying females resulting in greater use of more abundant non-fruiting milkweeds as oviposition sites. Their ability to develop vegetative plant tissue partially compensates for the poorer success in colonizing seed crops. Similarly, in areas of sympatry-competetion among species of *Spilostethus* and *Caenocoris nerii* for C. *gigantea* may have led to divergence in the utilization of this resource, with *S. hospes* being capable of developing on vegetative parts of the plant. Population studies of *L. equestris* showed that the amount of sunshine increased the population density of the bug positively but decreased seed production of the host and that during the year of increased seed density, the bug density was low but favoured build up during the following year. This phenomenon has lead to

TABLE 4. Simple correlation and regression coefficients for the population of *S. hospes*.

Year	Maximum temperature			Minimum temperature			Relative humidity			Rainfall		
	I	II	III	I	II	III	I	II	II	I	II	III
Host: *C. gigantea*												
Correlation coefficient (r)												
Total	0.79*	0.50**	0.35	0.54*	0.18	–0.54*	–0.63	–0.38	–0.03	–0.38	–0.51	–0.47**
Nymph	0.61*	0.37	0.44**	0.45**	0.30	0.56*	–0.66*	–0.04	0.10	–0.34	–0.01	–0.49*
Adult	0.81*	0.50**	0.35	0.55**	0.16	0.53*	–0.62	–0.42**	–0.25	–0.38	–0.02	–0.47
Regression Coefficient (b)												
Total	23.35	13.89	2.69	16.59	5.11	7.36	–12.69	–5.63	–0.20	–0.22	–0.30	–0.66
Nymph	2.15	1.41	0.27	1.63	1.15	0.61	–1.60	–0.08	–0.07	–0.02	–0.00	–8.63
Adult	21.20	12.48	2.44	14.96	3.96	6.75	–11.09	–5.56	–0.01	–0.20	–0.00	–0.71
Host : *V. cinerea*												
Correlation coefficient (r)												
Total	–0.52*	–0.82*	–0.47**	–0.59*	–0.66*	–0.39	0.36	0.09	0.14	0.14	–0.02	–0.27
Nymph	–0.52*	–0.73*	–0.41**	–0.56*	–0.52*	–0.32	0.37	0.01	0.09	0.20	0.03	–0.24
Adult	–0.50*	–0.81*	–0.53*	–0.62*	–0.75	–0.47	0.34	0.19	0.22	0.06	–0.15	–0.28
Regression coefficient (b_{yx})												
Total	–12.49	–23.72	–9.67	–14.75	–19.38	–14.52	5.94	1.39	3.29	0.07	0.00	–1.29
Nymph	–7.45	–13.71	–5.36	–8.26	–9.98	–7.67	3.63	0.12	1.31	0.06	0.01	–0.76
Adult	–5.04	–10.01	–4.31	–6.49	–9.41	–6.85	2.32	1.26	1.97	0.01	–0.04	–0.53

* Significant at 1% level.
** Significant at 5% level.

TABLE 5. Multiple regression analysis of the population of *S. hospes*.

Host : *C. gigantea*
Regression equation : population = —3.47 + 0.25 * maximum temperature + —0.19 * minimum temperature + 0.02 * relative humidity + —1.26* rainfall.

Source	SS	df	MS	F	P
Total	8.555	22			
Regression	3.244	4	0.811	2.749	0.060
Max. temp.	2.010	1	2.010	6.815	0.017*
Min. temp.	1.074	1	1.074	3.640	0.072
R.H.	0.006	1	0.006	0.020	0.887
Rainfall	0.153	1	0.153	0.521	0.479
Error	5.310	18	0.295		

Host : *V. cinerea*

Regression equation : population = 5.61 + —0.05* maximum temperature + -0.02* minimum temperature + -0.01* relaive humidity + 3.69.* rainfall.

Sources	SS	df	MS	F	P
Total	2.508	22			
Regression	0.955	4	0.238	2.767	0.059
Max. temp.	0.908	1	0.908	10.524	0.004**
Min. temp.	0.007	1	0.007	0.091	0.765
R. H.	0.026	1	0.026	0.303	0.588
Rainfall	0.013	1	0.013	0 151	0.701
Error	1.553	18	0.086		

* Significant; ** Highly significant.

considerable inertia in the temporal tracking of food resource by *L. equestris* (SILLEN-TULLBERG & SOLBRECK, 1990).

Abiotic factors also have an effect on the population of *S. hospes*. KIRKPATRICK (1923) believed weather directly or indirectly affected the population of *O. hyalinipennis* (Costa) in Egypt. He singled out rainfall as being responsible for considerably affecting the mortality, while WILLCOCKS & BAGHART (1937) opined heavy mortality to be due to sudden and marked changes in the prevailing weather. Analysis of the role of temperature, relative humidity and rainfall on the population fluctuation revealed interesting information on the colonization of *S. hospes* on the host plants. The build up as well as the establishment of the population on the

host plants was gradual although the decline was sudden. Under conditions of high maximum and minimum temperature as well as high relative humidity, greater colonization of the bugs occurred on *C. gigantea*, while decreasing temperatures and increasing relative humidity resulted in their colonization on *V. cinerea*. It is evident from Figures 1 and 2 that during the months when the population established itself on *C. gigantea*, only a low population was evident on *V. cinerea* and vice versa. This movement appears to be due to the availability of the food plants. The colonization of *S. hospes* on *V. cinerea* was observed only when the host plant springs up immediately after the monsoons. With the decreasing density of *V. cinerea* seeds, the bugs move to neighbouring *C. gigantea*. The relationship between the population on the abiotic environmental factors was statistically analysed using simple and multiple correlation and regression analysis. For the population of *S. hospes* on *C. gigantea* a definite positive correlation was observed for the total nymphal and adult populations with the maximum and minimum temperatures and no correlation observed with the other abiotic factors. The low negative correlation observed with the rainfall only in the third year appears negligible. A negative correlation with the maximum and minimum temperatures was observed for the population of *S. hospes* on *V. cinerea* and no correlation was evident with either the relative humidity or rainfall (Table 4). Table 5 provides the multiple regression analysis of the population data in relation to environmental factors. On both the host plants, maximum temperature showed to be a significant factor affecting the population of *S. hospes*. The drift in the population density among the host plants is the result of the problem of resource tracking besides the weather factors.

On the basis of the present findings on *S. hospes*, some conclusions can be drawn regarding the mechanisms that seem important in the population regulation of this bug. In addition to the environmental temperatures, food resources seem to be a limiting factor; not only the absolute amount of food is important, but also the distribution in time and space of these resources, and probably also the quality of the different foods. KIRITANI & SASABA (1969) found the relative abundance and seasonal combination of host plants were important in the regulation of the population of the bug, *Nezara viridula*. The competition with other insects for food resources may also be important for *S. hospes*. The action of weather seems to have effects on the pattern of food resource and the ability of the bug to keep pace with them. As earlier mentioned, weather conditions strongly affect the amount and distribution of the bug's food resources which in turn affects the reproductive potential of *S. hospes*.

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SPECIES OF *HETERODERES* LATREILLE FROM NORTH-WEST INDIA (CONODERINAE, ELATERIDAE: COLEOPTERA)

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Seven species of *Heteroderes* **viz.,** *H. sericeus*, *H. lenis*, *H. flavonotatus*, *H. algirinus*, *H. amaculatus*, *H. tubularis* **and** *H. farus* **have been reported from North-West India. Of these, last three are new and they have been described in detail, whereas, for known species only the synonyms, material examined and distribution have been given.**

(*Key words:* *Heteroderes*, pubescence, punctation, agricultural pests)

The genus *Heteroderes* was erected by Latreille (1834) on the basis of type species *Heteroderus fuscus* Latreille. It is characterised by the presence of two types of pubescence, short and long, and double punctation. These characters together with flat or convex head and entire lateral margin constitute the generic characters of *Heteroderes*.

106 species have been reported from all over the world of which 10 are from India (Schenkling, 1927). The present article is based on seven species of *Heteroderes* collected during five year's survey work of North-West India. Of these, three are new. These are the pests of crops and vegetables.

DESCRIPTION OF SPECIES

KEY TO THE SPECIES OF GENUS *HETERODERES* LATREILLE

1. Posterior angles of pronotum bicarinate: fourth tarsomere with broad lamellae, easily visible dorsally 2

   Posterior angles of pronotum unicarinate; fourth tarsomere with narrow lamellae below, not easily visible dorsally 3

2. Body reddish brown; scutellar surface convex .... *sericeus* Candeze

   Body black; scutellar surface flat ...... *lenis* Candeze.

3. Pronotum with a median acuminate projection in its posterior half 4

   Pronotum without any acuminate projection in its posterior half 5

4. Prothorax longer than broad; acuminate projection laterally compressed at base .... *flavonotatus* Bohemann

   Prothorax as long as broad; acuminate projection circular at base .... *amaculatus* sp. nov.

5. Body yellowish brown; frons with arcuate anterior margin .... *algirinus* Lucas

   Body dark brown; frons with straight anterior margin 6

6. Head flat, longer than broad .... *tubularis* sp. nov.

   Head convex, as long as broad .... *farus* sp. nov.

Heteroderes sericeus Candeze

Material examined – INDIA: Uttar Pradesh: 1 male; Wajuala (1400 m. asl, Almora); 11. viii. 1987; in agriculture field.

Distribution: India.

Heteroderes lenis Candeze

Material examined: INDIA: Uttar Pradesh: 2 males, 1 female; Bastia (600 m. asl,

Pithoragarh); 30.v.1986; under light in forest. 1 male, 1 female; Boom (500 m. asl, Pithoragarh); 30.v.1986; under light in forest. 5 males, 8 females; Pantnagar (300 m. asl); 1.vi.1986; under light. 3 males, 5 females; Kotdwar (396 m. asl); 4.vi.1986 under light in forest. 4 males, 3 females; Kaphara 1136 m. asl, Almora); 15.viii.1987; on leaves of *Colocassia.* 2 females; Mau (190 m. asl, Allahabad); 16.ix.1989; under light. 1 male; Dehra Dun (350 m als.); 29.viii.1988; under light. Haryana: 1 male 2 females; Chachhrauli (300 m. asl); 30.vii.1986; in agriculture field. 3 males, 2 females; Kurukshetra (250 m. asl); 15.vii.1987; under light. Jammu & Kashmir : 1 male ; Hiranagar (500 m. asl); 16.vi.1989; under stones in barren land. Himachal Pradesh: 1 male, 1 female; Paonta Sahib (600 m. asl); 20.ix.1986; under light.

Distribution : India.

Heteroderes flavonotatus Bohemann

Heteroderes flavonotatus Bohemann, 1851, Ins. Caffr. I, 2 : 407; *H. flavonotatus* Candeze, 1859, Mon. II: 362, *H. flavonotatus* Fleutiaux, 1919. Voy. Alluaud & Jeannel Afr. or col. XIII : 62.

Material examined: INDIA : Uttar Pradesh : 5 males, 4 females; Srinagar (750 m. asl); 20.vi.1986; in potato field. 1 male, 2 females; Garjia (300 m. asl, Ramnagar); 20.v.1986; in agriculture field. 4 males, 3 females; Rameshwar (500 m. asl, Pithoragarh); 25.vi.1986; under stones in barren hand. 2 males, 3 females; pantnagar (300 m. asl); 2.vi.1986; under light. 1 male, 2 females; Dehra Dun (350 m asl); 21.ix.1986; in agriculture field, 3 males, 1 female; Baghpat (250 m.asl); 30.ix.1987; in agriculture field. 1 male, 2 females; Rampur (200 m. asl); 23.ix.1987; in agriculture field. 1 male, 1 female; Mainpuri (200 m asl); 28.ix.1987; under light. 1 male, 2 females; Muzaffarpur (200 m. asl); 22.ix.1987; in cabbage field. 2 males, 2 females; Mirzapur (190 m. asl); 17.ix.1989; in tomato field, 2 males, 2 females; Deoria (250 m. asl); 25.ix.1989; in brinjal field. 1 male, 1 female; Mau (190 m. asl, Allahabad); 16.ix.1989; under light. 1 male; Haridwar (500 m. asl); 10.iv. 1989; in vegetable field. Haryana : 1 male, 1 female; Kala Amb (500 m. asl); 29.viii. 1989; under light. 3 males, 4 females; Chachhrauli (300 m. asl); 21.ix.1986; in agriculture field. Himachal Pradesh : 1 male, 2 females; Paonta Sahib (600 m. asl); 20.ix. 1986; under light. Rajasthan : 1 male, 2 females, Sawai Madhopur (200 m. asl); 19.iv.1990; under light.

Distribution: South Africa, India.

Heteroderes amaculatus sp. nov. (Photo. 1, Fig. 4).

Body brown, pubescence yellowish brown. Frons slightly convex with anterior margin arcuate. Antennae brown. Prothorax as long as broad, posterior angles unicarinate, pronotum with a small median acuminate projection in its posterior half with circular base. Legs brown, fourth tarsomere with narrow lamellae, not visible dorsally.

Measurement : Length 6.0 mm; Breadth 2.6 mm.

Head: Brown, broader than long (length 1.2 mm; breadth 0.9 mm), surface slightly convex; pubescence yellowish brown, dense; punctation double, dense, small punctures intermixed with larger ones; frons slightly convex with anterior margin arcuate. Antennae brown, second segment distinctly shorter than third.

Thorax: Pronotum brown, as long as broad (length 2.6 mm; breadth 2.6 mm), surface convex, sides gradually narrow anteriorly; punctation double, dense, small punctures on the interspaces between large rounded

Fig 1. *Heteroderes amaculatus* sp. nov.
Fig. 2. *Heteroderes tubularis* p. nov.
Fig. 3. *Heteroderes farus* sp. nov.

punctures; pubescence yellowish brown dense; posterior angles directed backward, unicarinate, a small median acuminate projection with circular base in the posterior half of pronotum. Scutellum brownish with black margin, subpentagonal, surface almost flat or slightly convex, anterior margin straight. Elytra brown, almost two and half times the length of prothorax, dorsal surface convex: pubescence yellowish brown, almost dense; striae punctate; interstriae convex or flat with double punctation; sides parallel at base, gradually narrow posteriorly. Legs brown, fourth

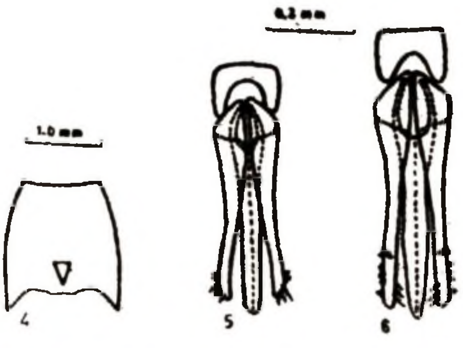

Fig. 4. Pronotum, *Heteroderes amaculatus* sp. nov.
Fig. 5. Male genitalia, *Heteroderes tubularis* sp. nov.
Fig. 6. Male genitalia, *Heteroderes farus*.

tarsomere lamellate below; lamellae narrow; concealed below fifth tarsomere, not visible dorsally.

Male genitalia: Male not available.

Holotype INDIA : Uttar Pradesh : 1 female on pin; Chhibramau (200 m. asl) 28.viii. 1986; in tomato field. **Paratype**–Mounted on pin. Haryana : 1 female; Chachhrauli (300 m asl) 30.vii.1986; in *bajra* field.

Distribution : India.

Heteroderes algirinus Lucas

Heteroderes algirinus Lucas, 1849. Explor. Algerie, Ins. II: 166; ***H. algirinus*** Rosenhauers, 1856. Thiere Andal.: 128 (*algerinus*)

H. algirinus Candeze, 1859, Mon. II : 368

H. algirinus Reitter, 1891, Wien Ent. Zeit. X: 147 (*Aeolus*)

Material examined – INDIA : Uttar Pradesh: 1 male, 1 female; Kalagarh (500 m. asl); 18.viii.1987; under light. 1 female; Etah (200 m. asl); 20.ix. 1987; in agriculture field. 1 male, 1 female; Shahjhanpur (200 m. asl); 27.ix.1987; under light. Haryana:

1 male; Kala Amb (500 m. asl); 29.viii.1986; under light.

Distribution: Japan, Algeria, India.

Heteroderes tabularis sp. nov. (Photo. 2, Fig. 5)

Body dark brown, pubescence yellowish. Head longer than broad, frons flat with anterior margin straight. Antennae light brown. Prothorax broader than long, without any projection, posterior angles unicarinate. Legs yellowish brown, fourth tarsomere with narrow lamellae, not visible dorsally. Aedeagus longer than parameres, tubular; parameres with inwardly curved posterior ends.

Measurement: Length 6.5 mm; Breadth 1.5 mm.

Head: Fuscous, longer than broad (length 1.0 mm; breadth 0.8 mm), surface flat; pubescence yellowish, dense; punctation double; frons flat with anterior margin straight. Antennae light brown, second and third segments subequal.

Thorax: Pronotum uniformly dark brown with anterior and posterior angles yellowish brown, broader than long (length 1.5 mm; breadth 1.7 mm), surface convex, sides slightly arcuate; punctation double, small punctures intermixed with large rounded punctures; pubescence yellowish, dense; posterior angles divergent, unicarinate. Scutellum yellowish brown with black margin, subpentagonal, declined anterirly, surface convex, anterior margin arcuate. Elytra uniformly dark brown, two and half times the length of prothorax, dorsal surface flat; pubescence yellowish, dense, striae punctate; interstriae flat with double punctation; sides parallel at base, gradually narrow posteriorly. Legs yellowish brown, fourth tarsomere lamellate below, lamellae narrow concealed below fifth, not visible dorsally.

Male Genitalia : Phallobase with stiaight anterior margin; parameres with posterior ends slightly curved inwards, without subapical process; aedeagus longer than parameres, uniformly tubular, apex rounded; furcae reaching the anterior margin of parameres.

Holotype : INDIA: Uttar Pradesh; 1 male on pin; Baghpat (250 m. asl); 17.vii.1986; in maize field. **Paratypes** – Mounted on pins. Uttar Pradesh: 1 male, 1 female; Pantnagar (300 m. asl); 2.vi.1986; under light. 1 male, 1 female; Sikandrabad (200 m. asl); 30.ix.1987; in potato field. 1 male, 1 female, Etah (200 m asl); 29.ix.1987; under light. Haryana: 2 males, 1 female; Kurukshetra (250 m asl); 23.ix.1987; in brinjal field.

Distribution : India.

Heteroderes farus sp. nov. (Photo 3, Fig. 6)

Body dark brown; pubescence yellowish. Head as long as broad, frons convex with anterior margin straight. Antennae light brown. Prothorax broader than long without any projection, posterior angles unicarinate. Legs yellowish brown, fourth tarsomere with narrow lamellae, not visible dorsally. Aedeagus longer than parameres, broad in middle.

Measurement : Length 6.0 mm; Breadth 1.6 mm.

Head: Fuscous, as long as broad (length 1.0 mm; breadth 1.0 mm), surface convex; pubescence yellowish, denser; punctation double, frons convex with anterior margin straight. Atennae light brown, second and third segments subequal.

Thorax : Pronotum dark brown with posterioi angles of lighter shade; broader than long (length 1.5 mm; breadth 1.7 mm), surface flat, sides arcuate; punctation double,

pubescence yellowish, dense, posterior angles divergent, unicarinate. Scutellum yellowish brown with black margin, subpentagonal, declined anteriorly, surface convex, anterior margin arcuate. Elytra uniformly dark brown, almost two and half times the length of prothorax, dorsal surface convex; pubescence yellowish, dense striae punctate: interstriae flat with double punctation; sides parallel at base, narrow posteriorly. Legs yellowish brown, fourth tarsomere lamellate; lamellae narrow, concealed below fifth, not visible dorsally.

Male genitalia: Phallobase with straight anterior margin; parameres straight posteriorly, without subapical process; aedeagus longer than parameres, broader in middle apex subconical; furcae reaching the anterior margin of parameres.

Holotype – INDIA : Uttar Pradesh : 1 male on pin; Baghpat (250 m. asl); 17.vii.1986; in maize field. **Allotype** – Uttar Pradesh : 1 female on pin ; Baghpat (250 m. asl); 17.vii.1986; in maize field. **Paratypes** – Mounted on pins. Uttar Pradesh : 2 males, 1 female; Baghpat (250 m. asl); 17.vii.1986; in maize field. Haryana : 1 female; Kurukshetra; (250 m asl); 15.viii.1987; in agriculture field.

Distribution : India.

DISCUSSION

The species of *Heteroderes* are mostly agricultural pests and are widely distributed from high ranges of Himalayas to the desert region of Rajasthan. Generally, they are of medium size and can be easily separated on the basis of morphological characters. The absence of subapical processes of the parameres is a character common to the species of *Heteroderes*. The male genitalia differ in the shape of aedeagus and of the distal ends of parameres.

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The authors are grateful to USDA, Washington and ICAR, India for financing the project on 'Systematics of Elateridae' of North-West India. Permission of the Forest Entomologist, FRI, Dehra Dun to compare the specimens with those housed in Entomological Museum is also gratefully acknowledged.

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BIO-ECOLOGY OF SIX SPECIES OF SYRPHID PREDATORS OF THE TEA APHID, *TOXOPTERA AURANTII* (BOYER DE FONSCOLOMBE) IN SOUTHERN INDIA[1]

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Studies were carried out on the life history and population dynamics of six species of syrphid predators viz., *Episyrphus balteatus* (De Geer), *Paragus tibialis* (Fallen), *Allobaccha nubilipennis* (Austen), *Betasyrphus serarius* (Wiedemann), *Dideopsis aegrota* (Fab) and *Ischiodon scutellaris* (Fab.) feeding on the tea aphid, *Toxoptera aurantii* (Boyer de Fonscolombe). At 26±2°C and 80±5% RH developmental - period of these predators, from egg to adult emergence, varied from 18 to 22 days. There were three larval instars and the maximum number of aphids were consumed by the seven/eight day old larvae. Population density of the predators was highest in September/October and January to March. These peaks almost coincided with the abundance of aphids in tea fields.

(*Key words:* bioecology, predatory efficiency, syrphids, *Episyrphus balteatus*, *Paragus tibialis*, *Allobaccha nubilipennis*, *Betasyrphus serarius*, *Dideopsis aegrota*, *Ischiodon scutellaris*, *Toxoptera aurantii*)

INTRODUCTION

Tea aphid, *Toxoptera aurantii* (Boyer de Fonscolombe) which is an ubiquitous pest of tea in southern India is more pronounced in fields recovering from pruning, thus leading to retarded shoot growth and delayed recovery of bushes. Larvae of aphidophagous syrphids are one of the important agents in the natural control of aphids (LAL & HAQUE, 1955; ROY & BASU, 1977; AGRAWALA & SAHA, 1986; SINGH & MISHRA, 1988). In the tea estates of southern India larvae of six syrphid species have been reported to be associated with the colonies of *T. aurantii* (MURALEEDHARAN & RADHAKRISHNAN, 1986). However, the information on the life processes of syrphids associated with the tea aphid is still lacking. The present studies were therefore undertaken to study the population fluctuations, life history and feeding efficiency of six species of syrphids associated with the tea aphid in the Anamallais (Coimbatore District, Tamil Nadu).

MATERIALS AND METHODS

Extensive surveys were undertaken in the tea growing regions of Kerala and Tamil Nadu. The places of collection in Kerala include Wynaad (Wynaad Dt.), Vandiperiyar, Peermade and Munar in Idukki District. In Tamil Nadu plantations of Nilgiris District and the Anamallais in Coimbatore District were examined for syrphid predators. From several fields, aphid infested tea shoots were collected with eggs, larvae and pupae of syrphids which were reared in the laboratory.

Seasonal fluctuations of *T. aurantii* and its syrphid predators were studied in the UPASI Tea Experimental Farm in the Anamallais (Coimbatore District), situated at an altitude of 1065 m above MSL. The tea bushes were spaced at 1.22 × 1.85 m

1 Part of the Ph.D. thesis of the first author.

and they were pruned in April 1984 at a height of 60 cm above the ground level. The experimental area was kept free from any pesticide application. Fortnightly observations on the aphid population, egg and larval count of syrphids were taken from tea shoots of twenty five randomly selected plants for four consecutive years from 1984 to 1987. The infested tea shoots were removed from each bush and were collected in polythene bags. The numbers of aphids and syrphid larvae present in each shoot were recorded.

Wooden framed mesh cages (60 × 40 cm) were used to study the life history of the syrphids. One pair of freshly emerged male and female was introduced into the cages. These were regularly provided with brushed tea flowers with 30 per cent honey solution as food and infested tea shoots for oviposition. Every day, the old shoots were replaced by fresh ones. Observations were made on incubation period, larval development, pupation, adult emergence and oviposition.

Glass chimneys (21 × 10 cm), the top of which were covered with nylon mesh were used to determine the efficiency of syrphid predators. Aphid infested tea shoots along with syrphid larvae were kept inside the chimneys and the number of aphids killed was determined by counting the carcasses every day.

Studies were conducted on the effect of food consumption of larval development, pupal weight and adult longevity of the four common and widely distributed species of syrphids viz., *E. balteatus*, *P. tibialis*, *A. nubilipennis* and *B. serarius*. From laboratory cultures, four batches (A,B,C and D) of newly emerged larvae of the four species were selected. Individuals of the four batches of syrphid larvae were provided with 10, 20, 30 and 40 aphids of almost of the same age. The cosumption was recorded by counting the number of live aphids on the next day. Simple correlations were made on the effect of aphid feeding on pupal weight.

RESULTS AND DISCUSSION

Larvae of six species of syrphids viz., *Episyrphus balteatus* (De Geer), *Paragus tibialis* (Fallen), *Allobaccha nubilipennis* (Austen), *Betasyrphus serarius* (Wiedemann) *Ischiodon scutellaris* (Fabricius) and *Dideopsis aegrota* Fab have been found to feed upon *T. aurantii* in the tea plantations of southern India. Among these, *E. balteatus*, *P. tibialis* and *A. nubilipennis* were common and widely distributed species. From the tea estates of southern India MURALEEDHARAN & RADHAKRISHNAN (1986) reported six syrphid species associated with the tea aphids which confirms the present findings. From the tea gardens of northeast India DAS (1974) reported five syrphid species feeding on *T. aurantii*. GHORPADE (1981) listed eleven syrphids preying upon *T. aurantii* in the Indian sub-continent.

During the period of study, maximum number of eggs and larvae of syrphids were observed during January to March and a smaller second peak was observed in August/September. Peaks in the populations of *E. balteatus*, *P. tibialis* and *A. nubilipennis* coincided with that of the aphids. However, *D. aegrota*, *B. serarius* and *I. scutellaris* were noticed in higher numbers only after the peak incidence of *T. aurantii*. Further, *D. aegota* was rarely seen in the experimental blocks in most of the months. Populations of all the predators were very low during the rainy months of June and July.

Under laboratory conditions, mating took place during flight or at rest. Copulation lasted for ten to fifteen minutes in the case

of *P. tibialis, I. scutellaris* and *B. serarius,* whereas the duration of mating was twenty to thirty minutes in *E. balteatus, D. aegrota* and *A. nubilipennis.* Males mated repeatedly and died one or two days after mating.

Females of *P. tibialis, A. nubilipennis, B. serarius* and *balteatus* started laying eggs after a pre-oviposition period of three days, whereas in *D. aegrota* and *I. scutellaris* the preoviposition period varied between three and four days. Eggs were deposited singly or in batches of two to five. In the field, most of the eggs were noticed on the second and third leaves of aphid infested shoots. The number of eggs laid by these syrphids significantly varied from species to species. Maximum number of eggs were laid by *E. balteatus* (25) followed by *P. tibialis* (13) and B. serarius (12). Fecundity rate was very low in *D. aegrota* (Table 1).

Eggs of *A. nubilipennis, B. serarius, D. aegrota* and *E. balteatus* were oval and white whereas that of *P. tiabialis,* they were cream coloured when freshly laid but turned brown before hatching. Incubation period in different species varied from two to four days.

Immediately after hatching, the larvae were pale white and changed to black in *E. balteatus, A. nubilipennis* and *D. aegrota,* whereas they were pale green in *I. scutellaris* and yellow in *P. tibialis.* There were three larval instars and the total duration of larva in different syrphid species ranged from 7 to 11 days (Table 1). Cannibalism was widely prevalent among the larvae of all the syrphid species. Larval period of all the syrphid species ranged between seven and eleven days and this is in conformity with the findings of BHATIA & SHAFFI (1933), LAL & GUPTA (1953), ROY & BASU (1977), SINGH & MISHRA (1988) and SHARMA & BHELLA (1988). Before pupation larvae stopped feeding and became inactive. Under the laboratory conditions larvae pupated on tea leaves or on the walls of glass chimneys.

Larvae started feeding right from the day of emergence and a gradual increse in aphid consumption was observed up to the sixth day in *P. tibialis* and seventh or eigth day in *A. nubilipennis, E. balteatus, B. serarius* and *D. aegrota* and sharply declined afterwards. Maximum number of aphids were consumed by the third instar larvae (Table 2). Among the six syrphids, *A. nubilipennis* and *D. aegrota* consumed the highest number of aphids followed by *E. balteatus, I. scutellaris* and *B. serarius.* Prey consumption was significantly low in *P. tibialis.* ROY & BASU (1977) reported that the larvae of *S. balteatus* and *I. scutellaris* consumed an average of 510 to 406.2 individuals of *Lipaphis erysimi,* which is high in comparison to the present study. This could be attributed to the difference in the aphid species offered as prey.

Food consumption directly affected larval duration and pupal weight. On a restricted diet of 10 to 30 aphids/larvae/day the larval priod was extended upto 13 days, whreas in normal course, the larval development would have been completed in nine days. Though, the rate of feeding did not affect the duration of pupal stage, it has a direct effect on the weight of pupae. In the restricted feeding experiments with an average consumption of 200 to 300 aphids/larvae, the pupal weight ranged form 9.00 to 12.00 mg. Increased consumption upto 40 aphids per day directly enhanced the pupal weight and larval duration. HAGVAR (1973) reported considerable increase in pupal weight for *Syrphus corollae* Fab. which fed on *Myzus persicae ad libitum.* Similarly, CORNELIUS & BARLOW (1980) also noted longer larval duration when aphid consumption was maximum. Present findings confirm the

TABLE 1. Life cycle of different syrphid species.

Species	Pre-ovi-position period (days ±S.E.)	Ovi-position period (days ±S.E.)	Number of eggs laid	Incubation period (days ±S.E.)	Duration of indicated larval instar (days ± S.E.)				Pupal period (days ±S.E.)	Adult Longevity (days ± S.E)	
					I	II	III	Total mean		Males	Females
Allobaccha nubilipennis	2.70± 0.20	3.40± 3.55	6.70± 1.22a	3.60± 0.16	3.00± 0.14	3.20± 0.19	3.20± 0.24	9.20± 0.19	10.60± 0.29	1.30± 0.15	6.50± 0.21
Betasyrophus serarius	2.70± 0.50	2.20± 0.34	11.60± 2.43b	2.80± 0.19	2.60± 0.16	2.80± 0.13	2.80± 0.24	7.70± 0.28	8.80± 0.44	1.60± 0.44	5.50± 0.16
Dideopsis aegrota	3.56± 0.17	0.89± 0.29	3.56± 0.17a	4.80± 0.17	3.33± 0.22	3.33± 0.22	3.44± 0.17	9.80± 0.31	10.67± 0.31	1.44± 0.15	5.70± 0.15
Episyrphus balteatus	2.80± 0.28	3.20± 0.59	22.40± 6.78c	3.50± 0.26	2.80± 0.19	3.70± 0.25	3.80± 0.19	9.10± 0.30	9.50± 0.38	1.30± 0.20	5.50± 0.16
Ischiodon scutellaris	3.00± 0.20	2.80± 0.40	7.60± 1.56ab	3.30± 0.28	2.90± 0.22	3.00± 0.14	3.40± 0.16	8.00± 0.21	9.50± 0.32	1.20± 0.13	5.60± 0.16
Paragus tibialis	2.58± 0.14	2.98± 0.32	11.75± 1.94b	3.80± 0.18	2.58± 0.14	2.50± 0.14	2.75± 0.14	6.70± 0.20	9.83± 0.48	1.25± 0.13	5.70± 0.20

CD : P = 0.01
4.41

TABLE 2. Predatory efficiency of the syrphids against *Toxoptera aurantii*.

Syrphid species	Mean (±SE) aphids consumption			
	I Instar	II Instar	III Instar	Total
Allobaccha nubilipennis	95.00± 27.38c	142.92± 30.35c	217.62± 38.76b	455.54± 94.55d
Dideopsis aegrota	108.40± 19.39c	140.60± 22 .77c	205.78± 25.62b	454.17± 66.96d
Betasyrphus serarius	51.32± 18.41b	100.74± 30.25bc	209.86± 34.88b	362.92± 83.79c
Episyrphus balteatus	48.52± 8.82b	109.58± 26.04bc	202.26± 45.71b	358.36± 79.88c
Ischiodon scutellaris	35.13± 8.50ab	66.00± 23.29ab	195.53± 48.53b	296.66± 78.52b
Paragus tibialis	20.90± 3.40a	44.73± 4.91a	94.78± 15.64a	160.41± 22.98a
CD: $P = 0.01$	19.73	44.48	59.64	51.43

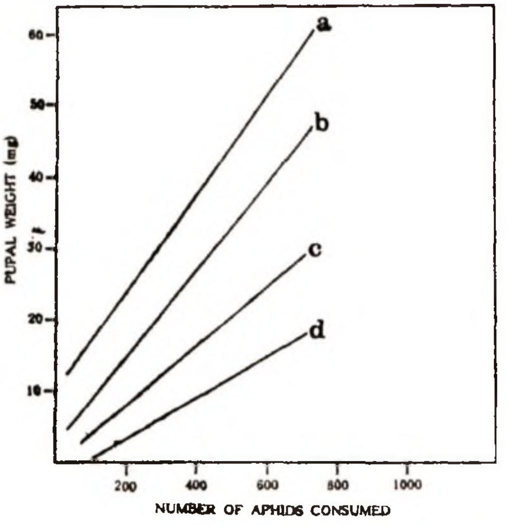

Fig. 1. Effect of food consumption on pupal weight in
(a) *Betasyrphus serarius* ($Y = 12.17 + 0.07X$, $r = 0.84$)
(b) *Allobaccha nubilipennis* ($Y = 4.19 + 0.06X$, $r = 0.91$)
(c) *Episyrphus balteatus* ($Y = 1.65 + 0.04X$, $r = 0.84$)
(d) *Paragus tibialis* ($Y=3.34 + 0.02X$, $r=0.91$)

earlier observations. When the larvae were fed with more than 400 aphids, the larval period was completed in 8 to 9 days and the pupal weight ranged from 25 to 36 mg. The study showed that food consumption had a positive relationship with larval duration and pupal weight in all the four syrphids (Fig. 1).

Larval and pupal stages of aphidophagous syrphids are parasitized by the members of nine families of Hymenoptera (CHAMBERS, 1988). But in the present study only larvae of *E. balteatus*, *A.* nubilipennis and *B. serarius* were found to be parasitised by *S. hakki*. Incidence of this encyrtid was more during September/October. Under field conditions, parasitism by *S. hakki* varied between 15 and 20 per cent.

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TWO NEW SPECIES OF THE GENERA *PISAURA* SIMON AND *TINUS* CAMBRIDGE (ARANEAE: PISAURIDAE) FROM INDIA

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Two new spider species of the genera *Pisaura* Simon and *Tinus* Cambridge (family Pisauridae) viz., *Pisaura bobbiliensis* sp. nov. from Bobbili, District Vijayanagaram, Andhra Pradesh and *Tinus chandrakantii* sp. nov from Pondicherry, Union Territory, India are described in detail and illustrated.

(*Key words:* *Pisaura bobbiliensis* sp. nov., *Tinus chandrakantii* sp. nov., Araneae, Pisauridae, India)

The first record of Indian pisaurid spider was made from India by Stoliczka in 1869 and thereafter through a series of publications of Cambridge (1985), Simon (1888, 1898), Thorell (1891, 1895), Pocock (1900) and Caporiacco (1935) who described as many as 13 species belonging to 9 genera. Recently Tikader (1970, 1977) from Sikkim and Andaman Island, Tikader and Malhotra (1976), Patel (1987) from Gujarat and Patel and Reddy (1990) from coastal Andhra Pradesh added another five species; four of *Pisaura* and one of *Tinus* from India. This makes the total number of 18 species belonging to 10 genera of Indian pisaurid fauna. The genus *Tinus* was recorded for the first time from Sikkim by Tikader (1970) and this is the second record from India.

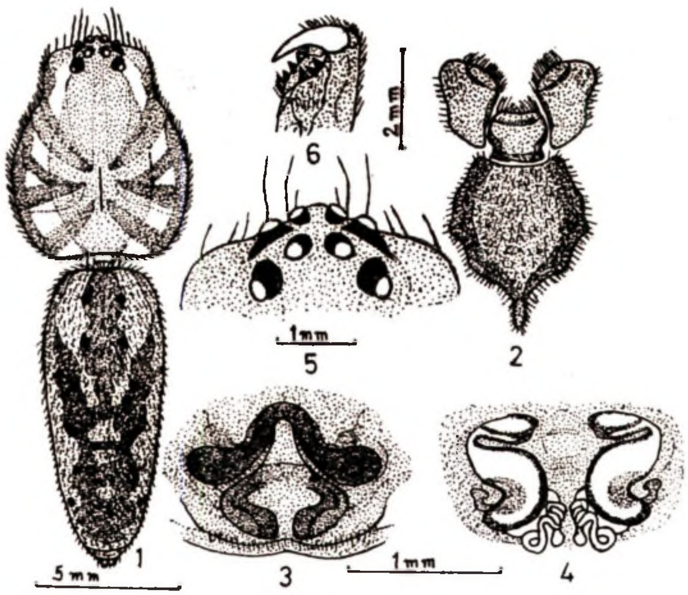
While examining the spider collections made by us (TSR) from coastal Andhra Pradesh and (BHP) from Pondicherry, we came across two new species of the genera *Pisaura* and *Tinus*, which are described and illustrated here. This makes the total of 20 pisaurid species from India.

The type specimens will in due course be deposited in the National Collections of Zoological Survey of India, Calcutta.

1. **Pisaura bobbiliensis** sp. nov. (Figs. 1–6):

General: Cephalothorax and legs light brown, abdomen brown. Total length 17.33 mm. Carapace 7.20 mm long, 5.86 mm wide; abdomen 9.73 mm long, 4.00 mm wide.

Cephalothorax: Longer than wide, convex, narrowing in front, clothed with thick fine hairs. Cephalic region is slightly high, middle of the cephalothorax provided with a longitudinal brown patch and centre of thorax with a conspicuous thin fovea. Eyes in two rows, anterior and posterior; anterior row short, slightly recurved, base of anterior lateral eyes having a black patch and equal in size. Posterior row strongly recurved, longer than the anterior row, bases of all the eyes encircled by black patches. Ocular quad rectangular and longer than wide as in Fig. 5. Clypeus narrow. Sternum heart shaped, very thinly pointed behind, clothed with spine like hairs. Labium and maxillae longer than wide, distal ends pale and clothed with scopulae. Sternum, labium and maxillae as in Fig. 2. Chelicerae strong, stout, inner and outer margins of fang furrow provided with three teeth each



Figs. 1–6. *Pisaura bobbiliensis* sp. nov.: 1. Dorsal view of female (legs omitted); 2. Sternum, labium and maxille; 3. Epigyne; 4. Internal genitalia; 5. Eyes as seen from above; 6. Right chelicera, inner view.

(Fig. 6). Legs reddish brown. Tibiae and metatarsi I and II provided with four and three pairs of ventral spines respectively; tibiae III and IV provided with three pairs of ventral spines; metatarsi III and IV with three ventral and four spines each on prolateral and retrolateral margins. Leg formula 1 4 2 3.

Male: Unknown

Abdomen: Longer than wide, yellowish brown, clothed with thick hairs. Mid-dorsally a lance shaped brown longitudinal patch is provided on the anterior part of abdomen, covered laterally by light coloured patches. Latreal margins provided with light yellow longitudinal band as in Fig. 1. Ventral side of abdomen pale in colour. Epigyne and internal genitalia are as in Figs. 3 and 4.

Holotype: One female in spirit.

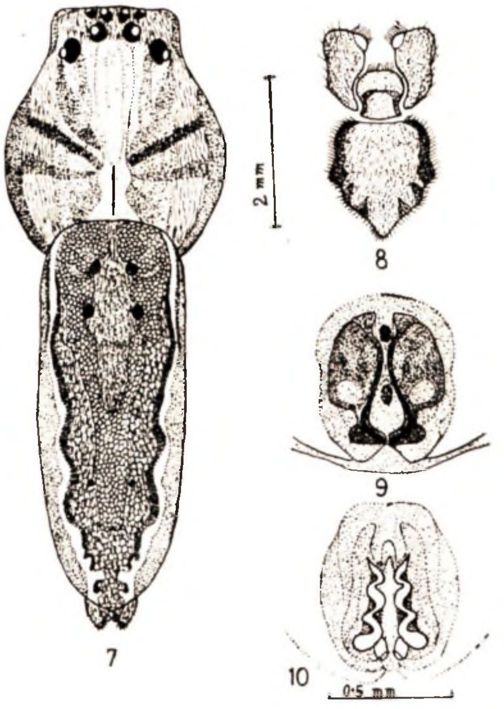
Type-locality: Bobbili, Dist. Vijayanagaram, coastal Andhra Pradesh, 25.ix.1985. Coll. T. S. Reddy.

Diagnosis: This species resembles *Pisaura gitae* Tikader but it is separated as follows: (i) Mid-dorsally a lance shaped brown longitudinal patch is provided on the anterior part of the abdomen covered laterally by light coloured patches and several very small dark brown circular patches, but in *P. gitae* mid-dorsally with a lance shaped brown longitudinal patch covered by two light coloured thin lined brackets arranged serially. (ii) Epigyne and internal genitalia are also structurally different.

2. **Tinus chandrakantii** sp. nov. (Figs 7-10):

General: Cephalothorax and legs orange coloured, abdomen yellowish brown. Total length 8.40 mm, carapace 3.45 mm long, 3.10 mm wide; abdomen 5.45 mm long, 2.00 mm wide.

Cephalothorax: Longer than wide, convex, cephalic region slightly high, narrowing in front, middle with a broad longitudinal



Figs. 7–10. *Tinus chandrakantii*: 7. Dorsal view of female (legs omitted); 8. Sternum, labium and maxille; 9. Epigyne; 10. Internal genitalia.

brown patch. Centre of thorax provided with a conspicuous long and thin fovea. Anterior row of eyes short, very slightly procurved, medians larger than the laterals and little closer to the laterals. Posterior row strongly recurved and longer than the anterior row, eyes nearly equidistant and equal in size. Ocular quad forms a trapaezium, broader behind than in front as in Fig. 7. Sterum heart shaped, pointed behind, pale in colour, clothed with hairs, near the base of corresponding coxae provided with deep brown patches. Labium longer than wide, basal portion highly constricted. Maxillae longer than wide; distal ends of labium and maxillae provided with distal scopulae. Sternum, labium and maxillae as in Fig. 8. Chelicerae strong and stout, inner and outer margins of fang furrow provided with three teeth each. Legs very long and strong, clothed with hairs and spines. Leg formula 1 4 3 2.

Male: Similar to the female and equal in size.

Abdomen: Longer than wide, pointed posteriorly and blunt anteriorly. Dorsum of abdomen mid-dorsally and laterally provided with longitudinal whitish stripe. Anterior mid-orsal side of abdomen provided with a lance shaped light brown patch as in Fig. 7. Ventral side pale in colour. Epigyne and internal genitalia as in Figs. 9 and 10.

Holotype: One female; *paratype* 6 females, *allotype* 2 males (subadults) in spirit.

Type-locality: Yanam, Pondicherry, Union Territory, 8.ix.1985. coll B. H. Patel.

Diagnosis: This species resembles *Tinus sikkimus* Tikader, but it is separated as follows: (i) Dorsum of abdomen mid-dorsally and laterally provided with longitudinal whitish stripes and covered by a brown stripe but in *T. sikkimus* dorsum with a longitudinal whitish stripe only. (ii) Epigyne and internal genitalia are also structurally different.

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STUDIES ON THE BUTTERFLIES OF SILENT VALLEY NATIONAL PARK[1]

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(*Received* 12 *March* 1992)

About 100 species of butterflies belonging to 9 families were collected in this study. The families Nymphalide and Papilionidae contained maximum number of species recorded here. Habitat preferences of the various groups of butterflies were also studied and five distinct biocoenoses with characteristic fauna were recognised, viz., interior forests, forest clearings and edges, forest canopies, grass lands and river banks. Of the various species collected, 13 were endemic to South India and which are now very much restricted in their distribution. This included 5 species having protected status.

(*Key words:* butterflies, Silent Valley, Kerala, India)

INTRODUCTION

The role of insects in the maintenance of essential life support systems in natural habitats is well recognized. Insects are very important for the recycling of nutrients, soil regeneration and protection, cleansing of waters, pollination of phanerogamic plants as well as for the natural regulation of pest outbreaks. The tropical rainforests are known to be the richest in species diversity as compared to other terrestrial habitats. Unfortunately most of these forests are situated in underdeveloped or developing countries where these areas are under considerable pressure from human population.

Among insects, the butterflies are ecologically very important. The adults generally feed on nectar and are important as pollinators of flowering plants. The larvae which feed on foliage are the primary herbivores in the ecosystem and are important in the transfer of radiant energy fixed by plants and making it available to the other organisms in the ecosystem.

The Silent Valley National Park which forms the core area of the Nilgiri Biosphere Reserve is a typical humid tropical rain forest situated on a plateau, bout 100 m above mean sea level. It covers an area of 9000 ha and exhibits considerable variations in the floristic composition, physiognomy etc., mainly due to climtic, edaphic and altitudinal variations. Four types of vegetations are encountered viz., (a) west coast tropical evergreen forest (b) subtropical broad leaved hill forests (c) montane wet temperate forest and (d) grasslands.

Butterflies, as most other Lepidoptera, show distinct patterns of habitat associations. The nature of vegetation, humidity, sunshine, availability of water, etc. are factors that determine the survival of a given species in a particular habitat. Information on such habitat preferences will be very useful in developing appropriate conservation strategies for the various species in future. Information in this regard is presented in this paper.

1. KFRI Scientific Paper No. 236.

MATERIALS AND METHODS

Sampling of butterflies was done by sweeping with a hand net in representative habitats in the National Park. Collections were made from several places including Aruvampara, Pulipara, Kattuvaramudi, Kattimudi and Walakkad. Sampling was as per the belt transect method. In grasslands a transect of 50 m width was taken. But in other habitats this could not be followed on account of terrain. The insects collected were set and preserved and subsequently identified by reference to literature collections.

RESULTS

About 100 species of butterflies belonging to 9 families were collected in this study. Of these, the identity of 95 species could be confirmed so far. Others are in the process of identification. The insect so far identified are listed in Appendix I.

Maximum number of species collected belonged to the families Nyphalidae and Papilionidae. Some species were present only in certain seasons whereas some others were present throughout the year.

Five bioceonoses supporting a characteristic assemblage of butterflies are recognized in the Silent Valley National Park. A summary of species occurring in the various biocoenoses is given below:

Dense forests:

These are essentially species that prefer the shade and coolness of dense forests and they seldom venture out into the open. They are usually dull coloured to match the surroundings and generally subsist on over-ripe fruits or sappy exudation of trees or on the nectar from plants belonging to the lower strata. They do not fly to high elevations and are mostly confined to the forest floor. *Melanitis leda, M. phedima varaha, Ypthima* sp., *Mycalesis* sp. and *Lethe* sp. were present very abundantly throughout the year. In the case of *M. leda*, wet and dry season forms very different in their colour patterns were present causing confusion in species identification. All these species were found in well regenerating wet evergreen forests and were not very common in other habitats.

Canopies

Most of the species found at higher elevations were comparatively bigger in size and were beautifully coloured and adorned with markings of various shapes, resembling birds while in flight. The nyphalids *Parthenos sylvia virens, Vindula erota soloma, Cirrochroa thais thais*: the papilionids *Papilio budha* and *P. paris tamilana* and the danaid *Idea malabarica* were the common species found in this strata. Many such species were found to feed at the flowers of various forest trees or twiners, although occasionally they were also observed to come to lower levels to feed at the flowers of plants like *Clerodendrum viscosum*, or on over-ripe fruits found on the ground or for settling on the damp mud near streams. Butterflies frequently found in forest canopies are swift fliers. An exception to this was *Idea malabarica* (Nymphalidae) which was found to glide gracefully through the valleys. The latter was often found in large numbers in the evergreen patches near Panthanthode and Campsite areas in the National Park. Most of the species recorded here are now very much restricted in their distribution and are mostly confined to the evergreen habitats in the Western Ghats.

Forest clearing and forest edges:

The forest edges as well as clearings are occupied by species that prefer bright sunlight. Such species often tend to be

brighter in colouration and they subsist on nectar of vrious shrubby plants found growing in such locations. At Silent Valley, the openings are colonised by profuse growth of plants like *Clerodendrum viscosum, Blumea alata, Ageratum conizoides, Vernonia canisoides, Desmodium* sp., *Barleria* sp., etc. Most of the papilionids (*Papilio polytes thesus. P. polytes romulus, Pachliopta aristolochiae, P. hector*), pierids (*Appias indra, Ceppora nadina, Catopsilia* spp., *Eurema laeta, E.* sp. nr. *lacteola* and the nymphalids (*Hypolimnas missipus, H. bolina, Neptis* spp., *Moduza procris, Cethosia nietneri*) were the common butterflies found in this habitat. Aggregation of butterflies was also characteristic in this zone. *Appias* spp., *C. nadina, Catopsilia* spp., *Eurema* spp. etc. were the common species found gregariously.

Grasslands:

The grasslands in Silent Valley are very extensive in area and support several species of shrubby plants. In low level grasslands tall grasses like *Cymbopogon* sp., *Themeda* sp., and shrubs like *Wendlandia thyrsoidea* and *Zizyphus rugosa* are the most common plants. Frequenly weeds like lantana, *Chromolaena* sp., *Crotalaria* sp., etc. also occur in patches.

The above plants support a very characteristic assemblage of butterflies. Danaid butterflies like *Tirumala limniace leopardus, T. sepentrionis dravidarum, Danaus genuita,* the nymphalids *Euploea core, Vanessa cardui* and Pierids like *Eurema hecabe, E. brigitta* etc. were the species generally found in this region. Small scale population build up and local migration of *T. limniace, T. sepentrionis, D. genuita, E. core* and *Eurema* spp. were observed during February-April, 1988.

Banks of streams and rivers:

Butterflies found in this habitat are frequent visitors to wet mud or damp moss along the banks of streams and rivers. Such species generally hover above the streams, visiting flowers in the vicinity and aggregating on damp soil or excreta of wild animals, licking the water out of it. Lycaenids, some papilionids and nymphalids belong to this category. Swarming of butterflies belonging to a single or several species was also observed. *Jamides celeno, J. alecto, Udara akasa, Castalius rosimon, Caleta caleta* (Lycaenidae); *Graphium doson doson, G. sarpedon teredon* (Papilionidae); *Cyrestis thyodamas* and *Kaniska canace haronica* (Nymphalidae), were the common species found in this habitat.

DISCUSSION

The study has indicated that the lepidopteran fauna of Silent Valley is rich and diversified. Of about 300 species of butterflies reported from southern India, nearly all have been recorded from the adjoining Nilgiri area of this biosphere reserve. Of these about 100 species have been now collected from the Silent Valley National Park.

The fauna of Silent Valley and of the Nilgiri Biosphere Reserve is very specialised due to very complex ecological conditions produced as a result of interaction between the typical rainfall patterns, temperature and topographical features. The various specialised ecological zones found in Silent Valley support a characteristic fauna containing several endemic species. With the destruction of local habitats, the range of many species of butterflies is now very much restricted and several species are now limited to certain forest patches only. Sixty-six species of butterflies which are currently rare in their distribution (Appendix II) have been reported from the Biosphere area. This includes 25 species having protected status under the Indian Wildlife Act. Of these, 13 species (containing 5 species

of protected status) have been recorded from Silent Valley in this study.

The tropical rain forests which are the result of over 60 million years of evolution are the centres of much species diversity. Man induced disturbances are the main factors that affect the survival of many natural communities and although extinction of species is supposed to create diversity due to diversification and adaptation of the surviving ones, destruction of species is unlikely to generate very much diversity in the rain forest (Turner, 1984). The occurrence of a rich and diversified fauna in some parts of Nilgiri Biosphere Region including Silent Valley, is largely due to conservation of forests in this region (Larsen, 1987, 1988). Adoption of appropriate conservation strategies for the forest biome in this region is very essential in order to safeguard its rich genetic diversity.

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APPENDIX-I

List of butterflies collected from Silent Valley.

RHOPALOCERA (BUTTERFLIES)

Danaidae

Tirumala sepentrionis dravidarum Fruhstorfer

T. limniace leopardus Butler

Parantica nilgiriensis Moore

P. aglea aglea Stoll.

Danaus genuita genuita Cramer

Idea malabarica malabarica Moore

Nymphalidae

Hypolimnas missipus Lin.

H. bolina Lin

Euthalia sp.

Euploea core core Cramer

Parthenos sylvia virens Moore

Vindula erota soloma de Niceville

Moduza procris Cramer

Cyrestis thyodamas ganescha Kollar

Neptis hylas varmona Moore

Neptis perius perinus Fruhstorfer

Phalanta phalanta Drury

Cirrochroa thais thais Fb.

Cethosia nietneri mahratta Moore

Vanessa cardui Lin

Vanessa indica nubicola Fruhstorfer

Cupha erymanthis maja Fruhstorfer

Ariadne merione Cramer
Junonia hierta Fb.
J. lemonias vaisya Fruhstorfer
J. almana Lin.
J. atlites Lin.
Kaniska canace haronica Moore
Hesperiidae
Tagiades litigiosus Moschler
Celaenorrhinus leucocera (Kollar)
C. ambareesa (Moore)
Potanthus pava pava Fruhstorfer
P. palnia Evans
Taratrocera sp.? *ceramas* (Hewutson)
Telecota sp.? *acigias*
Caltoris canaraica Moore
Lycaenidae
Cheritra freja (Fabricius)
Jamides celeno (Cramer)
J. alecto (Felder)
Jamides sp.
Udara akasa Horsfield
Celestrina lavendularis Moore
Castalius roskmon (Fabricius)
Caleta caleta Hewitson
Curetis sp.? *thetis* Drury
Arhopala centaurus
A. amantes (Hewitson)
Riodinidae
Abisara echerius Stoll.
Papilionidae
Troides minos Cram.
Chilasa clytia Lin.
Pachliopta pandiyana Moore
P. aristolochiae sp nr. *sawi* Evans
P. aristolochiae goniopeltis Roths.
P. aristolochiae f. *aristolochiae* Fb.
P. hector Lin.
Papilio polymnestor parinda Moore
P. paris tamilana Moore
P. Felenus Lin.
P. budha Westwood
P. liomedon Moore
P. demoleus demoleus Lin.
P. polytes thersus Cramer
P. polytes romulus Cramer
Graphium sarpedon teredon Felder
G. doson doson Felder
G. agamemnon agamemnon Lin.
Satyridae
Melanitis leda Lin.
M. phedima varaha Moore
Elymnias caudata Butler
Ypthima sp? *Ceylonica* Hewitson
Ypthima sp.
Mycalesis patnia Moore
M. igilia Fb.
M. anaxias Hewitson
Zipactis saitis Hewitson
Lethe rohria yoga Fruhstorfer
L. rohria neelgheriensis Guerin
L. europa Fabricius
Pieridae
Appias paulina galene Felder
A. lagela (Moore)
A. libythea
A. indra Moore
Delias eucharis Drury
Cepora nadina? *cingala* Moore
Cepora sp.
Eurema blanda Boisduval
E. hecabe Lin.
E. laeta Boisduval
E. brigitta Stoll.
E. sp. nr. *lacteola* Dist.
Catopsilia pomona Fb.
C. florella
C. pyranthe
Catopsilia sp.
Leptosia nina (Fabricius)
Libytheidae
Libythea myrrha Godart

APPENDIX - II

List of some rare butterflies recorded from the Nilgiri Biosphere Reserve.[1]

Family/Species	Species recorded specifically from Silent Valley in the present study (*)	Remarks
Papilionidae		
Chilasa clytia clytia Lin.	*	Protected Sch. I
Troides minos Cramer	*	
Pachliopta pandiyana Moore	*	
Papilio liomendon Moore	*	Protected, Sch. I
P. dravidarum Woodmason		
P. budha Westwood	*	Unable to survive in distrubed forests
Graphium doson eleias Fruh.		Evergreen forests
Pathysa antipathes alcibiades Fb.	*	Wettest rain forest
Pieridae		
Cepora nadina remba Moore		Wettest rain forest
Colias nilagiriensis Feld. & Feld.		South Indian endemic
Celatoxia albidisca Moore		Montane Sholas
Prioneris sita Feld. & Feld.		Protected, Sch. IV
Appias indra shiva Swinhoe		Protected, Sch. II
A. libythea libythea Fb.		Protected, Sch. IV
A. lyncida latifascia Moore		Protected, Sch. II
A. albina darada Feld & Feld.		South Indian endemic
Lycaenidae		
Arhopala canaraica Moore		Rare
A. abseus indica Riley		Rare
Zinaspa todara todara Moore		Scarce
Spindasis abnormis Moore		Scarce
Castalius rosimon rosimon Fb.	*	Protected Sch. I
Rachana jalindra macarita Fruh.		Very rare
Chilaria othona othona Hewit.		Rare
Rapala lankana Moore		
Tarucus callinara Butl.		Protected, Sch. II
Lampides boeticus Lin		Protected. Sch. II

1 Ref. Larsen, 1987, 1989

Nacaduba pactolus		Protected, Sch. II
Spindasis elima elima Moore		Protected, Sch. II
S. lohita Moore		Protected, Sch. II
Pratapa deva Moore		Protected, Sch. II
Tajuria cippus cippus (Fb)		Protected, Sch. II
Danaidae		
Parantica nilgiriensis Moore	*	Rare
Idea malabarica Moore	*	Rare
Satyridae		
Zipactis saitis Hewit.	*	Wettest rain forest Protected, Sch. II
Mycalesis anaxias anaxias Hewit.		Protected. Sch. II
Amathusinae		
Discophora lepida lepida Moore		Wettest rain forest
Nymphalidae		
Cirrochroa thais thais Fb.	*	Wettest rain forest
Cethosia nietneri mahratta Feld	*	Wettest rain forest
Dolenchthia hiaettide malabarica Fruh.		Rare
Hypolimnas missippus Lin.	*	Protected, Sch. I
Neptis soma palnica Eliot		Very rare, wettest rainforest Protected, Sch. II
N. columella nilgirica Moore		Very rare, Wettest rainforest
Parthenos sylvia Moore		Protected, Sch. II
Euthalia telchinia Men.		Protected, Sch. II
Gerosis bhagava bhagava Moore		Rare
Sarangesa dasahara davidsoni Swinhoe		Rare, in lowland forest
Tapena twaithesi twaithesi Moore		Rare, in lowland evergreen forests
Odontoptilum angulata angulata Feld. & Feld.		Rare, in lowland forests
Polyura schreiber wardii Moore		Very rare, wettest rain forest
Caprona alida vespa Evans		Very rare
Aeromachus pygmaeus Fb.		Limited to W. Ghats
Sovia hyrtachus de Niceville		S. Indian endemic
Thoressa honorei de Niceville		S. Indian endemic
T. sitala de Niceville		S. Indian endemic
T. astigmata Swinhoe		S. Indian endemic
T. evershedi Evans.		S. Indian endemic

Udaspes folus Cramer	Rare
Arnetta mercara Evans	Endemic to W. Ghats
A. vindhiana nilgiriana Moore	Endemic to S. and Central India
Cupitha purreea Moore	Scarce
Quedara basiflava de Niceville	Rare, S. Indian endemic
Oriens concinna Elwes & Edwards	S. Indian endemic found in montane & sub tropical forests
Potanthus pallida Evans	Rare
Pelopidas subochracea subochreacea Moore	Rare
Polytremis lubricans lubricans Herr. Schf.	Rare, found in wettest evergreen forests
Caltoris canaraica Moore	S. Indian endemic

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PRELIMINARY STUDIES ON MANGO LEAF COATING MITE, *CISABEROPTUS KENYAE* KEIFER (ACARI: ERIOPHYIDAE)

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(*Received* 30 *September* 1991)

The mango leaf coating mite, *Cisaberoptus kenyae* Keifer (Acari: Eriophyidae) has become a new pest of mango in Uttar Pradesh, with symptoms of white coating on the leaves. Studies on the suitable sample size., monthly population fluctuation, susceptibility of some common varieties and per cent leaf area coated in these varieties by *C. kenyae* were conducted in Varanasi, India. The results revealed that sample size 2 sq. mm was better suitable for estimating the population of this mite. The population of *C. kenyae* increased from November and reached a peak in March and minimum in December. Severity of incidence varied with variety. Among five varieties 'Himsagar' was most susceptible and 'Chowsa' was least susceptible. Percentage of leaf surface area coated was most in the variety 'Himsagar' and least in the variety 'Deshi'.

(*Key words:* *Cisaberoptus kenyae*, population fluctuation, varietal susceptibility)

INTRODUCTION

Cisaberoptus kenyae (Keifer) has become a new pest of mango in Uttar Pradesh. The mite was first described by KEIFER (1966) from Kenya. MOHANASUNDARAM (1983) reported the mite from South India for the first time. The feeding of this mite severely injures the epidermis tissue which turns into necrotic brown patches. The injured tissue mainly of mid rib exudes a milky substance which hardens into a white crusty membranous film covering different places infested by the mite. No information on its seasonal incidence and varietal susceptibility is available. The present study reports about the suitable sample size for estimating population of this mite, seasonal incidence, relative abundance and per cent leaf area coated by *C. kenyae* on five varieties of mango.

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MATERIALS AND METHODS

The study was carried out in Mango Orchard of the Institute of Agricultural Sciences, Banaras Hindu University, from November 1986 to October, 1987. Five varieties of mango viz., 'Deshi', 'Langra', 'Himsagar', 'Fazli' and 'Chowsa' were selected for sampling of mite at monthly interval. Samples of three leaves infested by mite *C. kenyae* were taken from each variety in polythene bags separately and brought to the laboratory. The population of the mite was counted under stereoscopic binocular microscope by scraping the whitish coating from the upper surface of the leaves. For determining suitable sample size for counting the population of *C. kenyae*, 40 observations from each quadrat of 2 sq. mm, 4 sq. mm and 6 sq.mm were taken. The number of mites in each quadrat were carefully counted and coefficient of variation and ratio of standard error of mean to

mean (SE $\pm$ M/M) were worked out to estimate the variation in mite population on different quadrat size. The per cent leaf area coated by mite was measured by drawing the total area covered by entire leaf and area covered by coating on to a cm graph paper. The meteorological data (temperature, relative humidity and rainfall) were recorded for the period of study from Meteorological section of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The effect of abiotic factors on the population of *C. kenyae* was determined by computing the simple correlation coefficient (r) of dependent (mite population) and various independent variables (abiotic factors, viz., temperature, relative humidity and rainfall). For testing the significance of simple correlation coefficient, 't' test was applied which is expressed as,

$$t = r / \sqrt{1 - r^2} \times \sqrt{n - 2}$$

where 'r' is the simple correlation coefficient and 'n' is the total number of observations.

RESULTS AND DISCUSSION

Selection of sampling size:

For correct enumeration of the population of mite, the efficiency of different sizes of sample must be ascertained as the accuracy will depend on the size and density of the population. In the present investigation, it is evident from Table 1 that maximum number of mite was collected from sample size 2 sq. mm and least number of mite was collected from sample 4 sq. mm. It is also evident from Table 1 that coefficient of variation and ratio of standard error of mean to mean is least at sample size 2 sq. mm statistically it is suggested that sampling unit area should be such that ratio of standard error of mean to mean (SE $\pm$ M/M) and coefficient of variation (CV) should be least. On the basis of least CV and least SE$\pm$M/M, the sample size of 2 sq. mm was found to be better suitable for estimating the population of *C. kenyae* and that was followed throughout the experiment.

Population fluctuation of C. kenyae *in different months:*

Table 2 gives the population fluctuation of *C. kenyae* in relation to three major abiotic factors in different months. The data were recorded from November 1986 to October 1987 but as the population of mite was not found during the period from July 1987 to October 1987, Table 2 shows the data recorded from November 1986 to June 1987. It is evident from Table 2 that maximum average population of 44.40 per sq. mm was found in the month

TABLE 1. Mean number of mite, SE $\pm$ M/M and Coefficient of variation at different quadrat size.

Size of quadrat (sq. mm)	Mean number of mite/sample (n = 40)	SE $\pm$ M/M	C.V
2	72.42 $\pm$ 9.88	0.107*	30.47*
4	48.37 $\pm$ 16.12	0.118	33.32
6	67.30 $\pm$ 22.99	0.121	34.16

* Least

TABLE 2. Seasonal incidence of *Cisaberoptus kenyae* in relation to three major abiotic factors in Varanasi from November 1986 to June 1987.*

Months	Average population of mite/2 sq. mm	Average temp. (°C)	Average RH (%)	Total rainfall (mm)
November 1986	28.20	21.89	45.97	—
December	23.60	17.40	65.50	7.4
January 1987	30.40	15.47	56.91	12.4
February	32.80	20.05	53.63	2.3
March	44.40	25.31	44.00	—
April	36.80	30.04	44.00	0.8
May	32.40	31.64	53.50	51.9
June	29.60	35.46	48.50	—

* Mite population was not found from July to October 1987.

Factors	Correlation coefficient (r)	
Average population vs. average temp.	.29	Significant at 1%
Average population vs. average RH	−.67*	level of significance
Average population vs. total rainfall	.20	

of March, whereas the lowest population of 23.60 per 2 sq. mm was recorded in the month of December. The population of mite started increasing from November and reached its peak during summer months and no population was found from July onward until October. A significant negative correlation was found between average relative humidity and population of mite. A relative humidity above 50% seemed to be detrimental for the mite. The population of mite was positively correlated with average temperature and total rainfall, but the correlation was not significant. In Israel, STRERNLICHT & GOLDENBERG (1976) found that high temperature (35°C) combined with low relative humidity (20%) during May caused 90 per cent mortality of *C. kenyae*. In the present investigation, a temperature around 25°C combined with relative humidity below 50% in the month of March was found to be favourable to the mite.

Relative susceptibility of different varieties of mango to C. kenyae:

Table 3 gives the population of *C. kenyae* on five varieties of mango. The average population of mite has been found to be maximum (36.5 per 2 sq. mm) on 'Himsagar', whereas, the minimum was

TABLE 3. Monthly average population of *Cisaberoptus kenyae* on different varieties on mango.*

Months	Mite population/2 sq. mm					Total	Average
	Deshi	Langra	Himsagar	Fazli	Cowsa		
November 1986	28	19	30	30	34	141	28.20
December	36	37	19	13	13	118	23.60
January 1987	21	36	28	32	35	152	30.40
February	31	38	40	34	21	164	32.80
March	51	46	59	45	21	222	44.40
April	39	44	31	27	43	184	36.80
May	39	42	40	19	22	162	32.40
June	25	21	45	38	19	148	29.60
Total	270	283	292	238	208	1291	259.00
Average	33.30	35.37	36.50	29.75	26.75	162.12	32.42

* Mite population was not found from July to October 1987.

Varieties C.D. (P = 0.05) = NS.

Periods C.D. (P = 0.05) = NS.

TABLE 4. White coating (%) on different varieties of mango leaf due to *Cisaberoptus kenyae*.

Months	White coating (%) on leaf on different varieties of mango					Total	Average
	Deshi	Langra	Himsagar	Fazli	Chowsa		
November 1986	24.44	13.71	25.96	24.44	37.22	125.77	25.15
December	19.49	30.66	12.87	12.12	19.10	24.24	18.75
January 1987	19.92	31.15	23.27	25.47	26.25	126.06	25.21
February	24.85	32.85	37.26	28.30	19.92	143.18	28.64
March	30.39	34.17	38.67	29.45	18.50	151.18	30.24
April	22.39	26.98	36.00	20.06	27.00	132.43	26.49
May	20.29	28.75	34.66	20.03	28.50	132.23	26.45
June	21.50	24.44	35.15	25.23	20.73	127.05	25.41
Total	183.27	222.71	243.84	185.10	197.22	1032.14	207.39
Average	22.91	27.84	30.48	23.14	24.64	129.64	25.92

Varieties C.D. (P = 0.05) = NS.

Periods C.D. (P = 0.05) = NS.

recorded on variety 'Chowsa' (26.75 per 2 sq. mm). However, the difference was found to be non-significant. RAMANI & HAQ (1989) while studying the distribution pattern of *C. kenyae* on five varieties of mango, found that abundance of *C. kenyae* varied significantly among the varieties tested. It is evident from the results of the present investigation that variety 'Himsagar' was more susceptible to attack of this mite than other varieties. The varieties 'Chowsa' was relatively less susceptible to this mite.

Percentage of white coating on different varieties of mango in different months:

Table 4 indicates the percentage of leaf area coated on different varieties of mango in different months. The percentage of leaf area coated by this mite was found to be maximum (30.23%) in the month of March whereas only 18.75% coating was found in December. Table 4 also indicates that variety 'Deshi' had less coating in comparison to other varieties. An average of 22.90 per cent of leaf area was found to be coated on variety 'Deshi', whereas maximum (30.48%) coating was found on the leaf of 'Himsagar'.

An overall analysis of informations of the present investigation indicates that all the five varieties studied are more or less susceptible to the mite infestation and the peak infestation of *C. kenyae* coincides with the time of flowering in mango (Feb-March) in Uttar Pradesh. Therefore, an intensive study would be needed to establish the impact of leaf coating due to *C. kenyae* on the flowering and fruiting in mango.

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A NOTE ON THE PATTERN OF ESTERASES OF TWO TERMITE SPECIES, *COPTOTERMES CEYLONICUS* AND *ODONTOTERMES WALLONENSIS*

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Esterase patterns of two termite species are compared. Substrate specificity and inhibitor sensitivity of the individual esterase bands is tested to classify them into different categories.

(*Key words:* termites, *Coptotermes*, *Odontotermes*, esterases)

Electrophoresis is an important tool in establishing taxonomic relationship among different animal species (WRIGHT, 1974). Although this technique has been used in resolving some complex taxonomic problems of some ant species (HALLIDAY, 1975; CROZIER, 1977; WARD, 1980; HEINZE, 1987), it has not yet been fully utilized for studying the relationships existing among the social insects. Termites are among the important social insects damaging the structural wood of domestic houses, crops and forests (SAMMAIAH, 1989). Taxonomic studies of termites are largely based on the head capsules and mandibular structures of the soldiers and a need exists for applying the biochemical tools for studying the taxonomic relationships of the related species (BAGINE, 1987). This paper is a preliminary report on the esterase patterns of two termite species collected from the campus area of Kakatiya University, Warangal. The workers and soldiers of *Coptotermes ceylonicus*, *Odontotermes wallonensis* and the queens of *Coptotermes ceylonicus* were used in the study. The termites were homogenized (20%) separately on 0.05M Tris-HCl buffer. The homogenates were centrifuged at 2000 rpm for 10 minutes at room temperature (28° C). The supernatants were mixed with an equal volume of 20% sucrose solution to which a crystal of bromophenol was added as a tracking dye. An aliquot of 0.1 ml of this mixture was used for electrophoresis. Tris (0.5M), EDTA (0.016 M), borate (0.65M) buffer (PH 8.3) was used as a gel buffer. The same buffer diluted (1:9) with distilled water was used as an electrode buffer. A simplified polyacrylamide (disc) gel electrophoresis was performed according to the procedures of LAKSHMIPATHI & REDDY (1989). Esterases were classified on the basis of the sensitivity to inhibition by physostigmine (10^{-4} M), Paraoxon (O, O-diethyl O-(4-nitrophenyl) phosphate; 2×10^{-5} M) and pCMB (Para chloro mercuribenzoate; 10^{-3}M) according to the procedures of HOLMES *et al.* (1968), HART & COOK (1976) HARITOS & SALAMASTRAKIS (1982); REDDY & LAKSHMIPATHI (1988) and LAKSHMIPATHI & REDDY (1989).

The patterns of esterases observed in the queen, workers and soldiers of *Coptotermes* and those of workers and soldiers of *Odontotermes* are presented in Fig. 1. The substrate specific properties and inhibitor sensitivity of the enzymes are presented in Table 1.

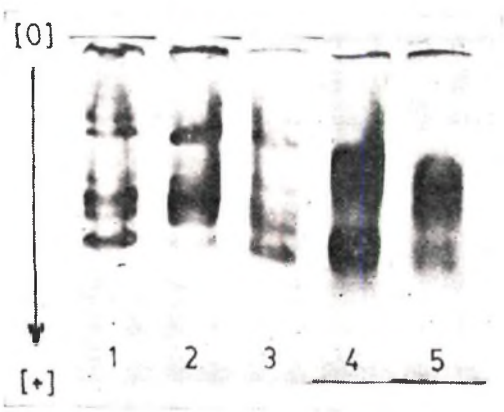

Fig. 1 Patterns of esterases of *Coptotermas ceylonicus* 1. Queen 2. Workers. 3. Soldiers and *Odontotermes wallonensis* 4. workers, 5. soldiers.

(O) Origin (+) Anode ↓ direction of current flow.

No differences were observed in the electrophoretic mobility of esterases of queen, workers and soldiers. There is, however, a difference in the activity of the zones between the different castes, as indicated by the variation in the intensity of the stain deposited on the gel. The queen and workers in *Coptotermes* and workers in *Odontotermes* exhibit higher activity over that of soldiers. The electrophoretic pattern exhibits interspecific variation. The substrate specificity and inhibitor sensitivity of esterases was studied in the workers and soldiers. *Coptotermes* exhibits six esterase active zones on the zymogram. All the zones hydrolyze the 1-naphthyl acetate, 2-naphthyl acetate and 1-naphtyl propionate. Zones

TABLE 1. Substrate specificity and inhibitor sensitivity of individual esterase zones observed in the workers of *Coptotermes ceylonicus* and *Odontotermes wallonensis*.

Species	A. *Coptotermes ceylonicus*					B. *Odontotermes wallonensis*		
Relative Mobility	56	50	44	40	26	22	56	34
Substrate specificity								
1-naphthyl acetate	+	+	++	+	++	+	+++	+++
2-naphthyl acetate	+	+	+	+	+	+	—	—
1-naphthyl propionate	+	+	++	+	+	+	++	+++
1-naphthyl butyrate	+	—	+	—	+	—	+	++
1-naphthyl laurate	—	—	—	—	—	—	+	+
Inhibitor sensitivity								
Physostigmine	+	—	—	+	++	+	+	+
pCMB	+	—	—	+	+	—	++	++
Paraoxon	+	—	—	—	—	—	—	+
Classification	ER	CHsp	CHsp	CE	CE	Esdp	CE	ER

+ + + high activity, + + moderate activity, + low activity, — no activity.

PCMB – Para chloro mercuribenzoate, Paraoxon - (O, O-diethyl O-(4-nitrophenyl) phosphate.

CE = Carboxylesterase, Esdp = Enzymes inhibited by paraoxon and pCMB, CHsp = Cholinesterase like enzymes, ER = Enzymes resistant to inhibitors.

1, 3 and 5 hydrolyze 1-naphthyl butyrate as well. None of the zones hydrolyze the laurate ester. Zone 1 is not affected by any of the three inhibitors. It is classified as an ER esterase (resistant esterases). Zones 2 and 3 are inhibited by all the three inhibitors. They are classified as cholinesterase like (CHsp) enzymes. Zones 4 and 5 are inhibited by the Organophosphate (Paraoxon) alone. They are classified as Carboxylesterase (CE). Zone 6 is inhibited by Paraoxon and pCMB. It is classified as Espd esterase. *Odontotermes* exhibits two zones on the zymogram. Both the zones hydrolyze the 1-naphthyl esters of acetate, propionate, butyrate and laurate but do not hydrolyze the 2-naphthyl acetate. Zone 1 (Rm 56) is a carboxylesterase while zone 2 is an ER esterase. Among the social insects ants were shown to exhibit constant patterns of esterases in developmental cycles, and no differences were found between the various castes of the same species; the patterns were, however, found to be species specific (HEINZE, 1987). The observations in the present investigation also indicates that like those of ants, esterase patterns in the two termites studied exhibit species specific variation but not caste specific variation. The substrate specificity and inhibitor sensitivity of individual zones also yielded identical results in workers and soldiers.

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